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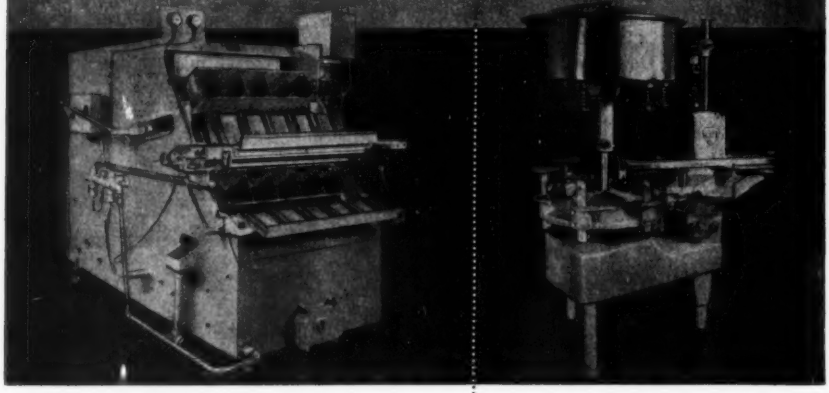

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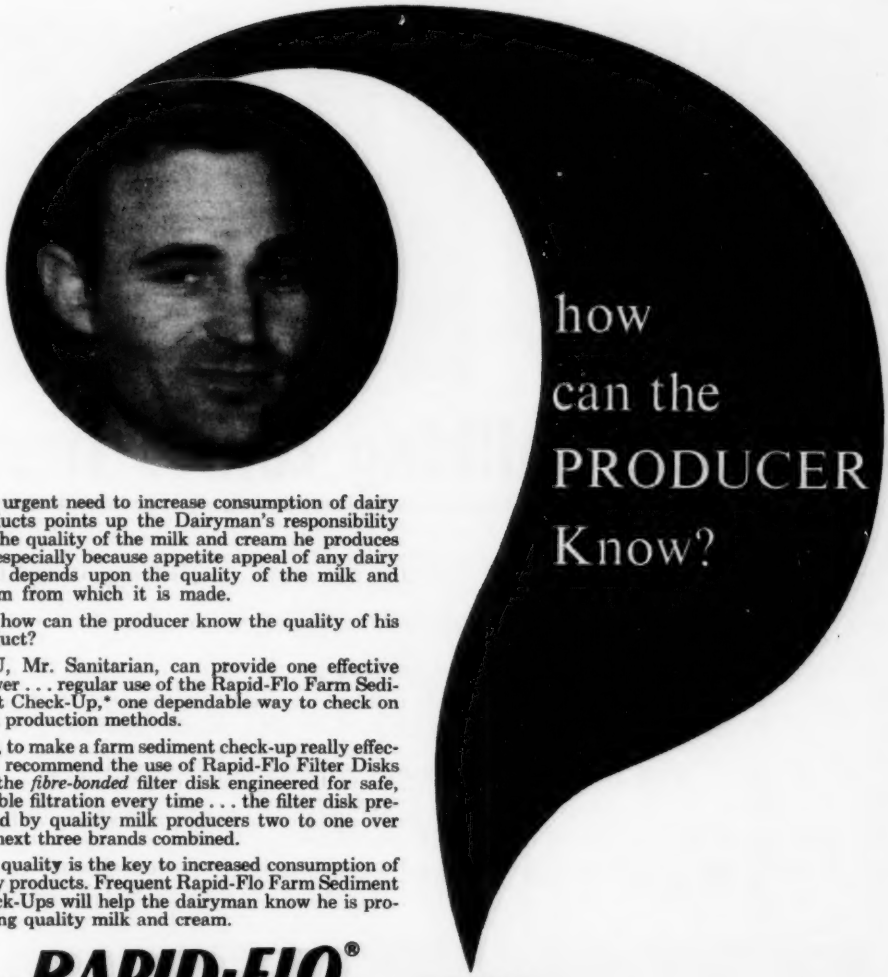

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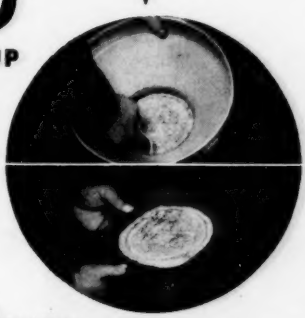

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CONSUMPTION AND DIGESTIBILITY OF LESPEDEZA SERICEA HAY AND ALFALFA HAY PLUS GALLOTANNIN[1]

G. E. HAWKINS, JR.

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Sericea lespedeza, *Lespedeza cuneata*, the basic forage crop on many dairy farms throughout the southeastern part of the United states, apparently is not a highly palatable roughage *(1, 2, 9, 18, 19, 24)*. Several investigations indicate that livestock will consume less dry matter *(1, 9, 16, 22)* and produce less milk *(9, 10, 12, 13, 24)* or make slower gains *(2, 16, 17, 22)* on sericea than on high-quality forages. Conversely, other reports, several of which have been reviewed by Morrison *(18)*, suggest that sericea is a high-quality forage. Apparently most immature sericea plants are acceptable to cattle, whereas more mature plants of this species are grazed with less relish or not at all *(2)*. The varied reports possibly are explained by differences in the percentage of infestation of the sericea pasture or hay with other forage species and by differences in the stage of maturity of the forages at the time of grazing or at the time of cutting for hay. Generally, however, it has been assumed that the tannin content of sericea has a pronounced effect on the consumption of this forage.

Several studies support the assumption that the tannin content of sericea may be responsible for the apparent low palatability of this legume. Olson and Elrod *(19)* found that legumes readily eaten by livestock, such as alfalfa, hop clover, and White Dutch clover, contained approximately 1.3% tannin, whereas sericea and other legumes not readily eaten by livestock averaged 5% tannin. Also, Clarke *et al. (5)* and Stitt and Clarke *(20)* found that the tannin content of sericea increased during the early summer. This increase in the tannin content of the sericea probably was associated with an increase in the stage of maturity of the plants. In addition, Donnelly *(6)* fed sericea with variable tannin content, cafeteria style, to cattle. Wilkins *et al. (23)* fed similar forage to sheep. Both types of livestock consumed more forage from the low-tannin lines of sericea than from the high-tannin lines. On the other hand, forage consumption by cattle *(11)* and concentrate consumption by rats *(15)* apparently were not affected by adding 5% of crystalline or powdered tannic acid to the diets. Also, samples of several lespedezas—Tennessee 76, Kobe, and Korean—were found to contain almost as much tannin as sericea *(15)*. Since these lespedezas generally

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[1] This study was conducted as part of a cooperative project with the Tennessee Valley Authority and was supported in part by funds supplied by T.V.A.

are considered palatable forages either for grazing or as hay, the significance of tannin in determining the palatability of sericea seems questionable.

Digestibility studies by Holdaway *et al. (13)* and McCullough *et al. (17)* indicate that the total digestible nutrient content and the coefficients of digestibility of dry matter and of crude protein of sericea lespedeza are relatively low as compared with corresponding values for alfalfa. The low digestibility of dry matter and of crude protein in sericea probably accounts for the differences in rate of growth or of milk production when sericea has been compared with other forages that provided more digestible protein per pound of dry matter consumed.

The present investigation was initiated to study the effects of tannin, per se, on consumption and on utilization of dry forage and to further characterize the nutritive qualities of sericea lespedeza, *L. cuneata*.

EXPERIMENTAL PROCEDURE

The study was divided into two parts, a palatability trial and a digestion trial.

Chemical analyses. The forages fed during the palatability and digestion trials were from the same cutting, and samples of the diets were collected at intervals throughout the feeding periods. Daily aliquots of the feces from steers on the digestion trial were dried at 60° C. Subsequently, the feed and fecal samples were ground to pass a 40-mesh screen and composited for analyses.

The chemical determinations included the tannin, crude protein, and lignin contents of the diets (Table 1) and the crude protein content of the feces. Crude protein was determined by the micro-Kjeldahl method *(4)* and lignin was measured by the method of Ellis *et al. (7)* and corrected for crude protein. Lignin was measured before and after extraction of tannin by refluxing for 1 hour. The class of tannin in each diet was determined by qualitative tests described by Gisvold and Rogers *(8)*, whereas the quantity of tannin was measured by the method of Valaer *(21)* with modifications that are in general use at this station. The modifications are as follows: *(a)* 0.5 g. of the finely ground plant sample was weighed into an Erlenmeyer flask and 250 ml. distilled water was added; *(b)* it was refluxed on a hot plate for 1 hour; *(c)* it was stoppered and allowed to stand over night; *(d)* to a 100-ml. volumetric flask containing 75 ml. of distilled water was added a 4-ml. aliquot of the aqueous extract. After development of color as described by Valaer *(21)*, the density of the solution was read on a colorimeter with filter No. 515.

Palatability trial. Six dairy calves approximately 6 months of age were placed in two groups of three calves each, according to size, and within groups were assigned at random to the test diets. The experimental design was a three by three Latin square, replicated twice, involving three diets and three 10-day experimental feeding periods. All calves were fed alfalfa hay during a 10-day standardization period. In the subsequent test periods the calves were fed the following diets: A, alfalfa hay; B, 95% alfalfa hay and 5% powdered tannic acid; and C, sericea lespedeza hay.

The alfalfa hay was cut at approximately one-tenth bloom and the sericea hay was cut at a height of approximately 15 in. Both hays were harvested in

May, 1952, and probably would have classified as U. S. No. 1, extra green and extra leafy. They had been in storage less than 9 months at the time of feeding. In order to mix diet B adequately and to provide a uniform material for consumption by the test subjects, both hays were ground to pass a ¼-in. mesh screen.

The calves were stanchioned except during weigh periods and were fed twice daily more hay than they would consume during the intervals between feedings. Water was offered to the calves at the morning and evening feedings. The amount of feed and of water consumed by each calf daily was recorded. Each calf was weighed at the beginning of each experimental period and at the end of the final period.

Digestibility trial. Three dairy steers, approximately 1 year of age, were fed controlled quantities of each of the three diets described for the palatability trial. The amounts fed daily ranged between 12 and 15 lb. for the three steers and were approximately 1 lb. less than the average amounts that each steer would eat. Digestibility of dry matter in the diets, by the three steers, was determined by the total collection method. In the digestion trial, after an 8-day preliminary feeding period, the feces were collected in toto for a 4-day period.

RESULTS

Chemical analyses. The mean tannin, crude protein, and lignin contents of the experimental diets are shown in Table 1. Sericea, diet C, contained more tannin and more lignin but less crude protein than alfalfa. The apparent lignin content of sericea was reduced approximately 45% by extraction of tannin from the samples and correcting for crude protein. Also, the apparent lignin value for diet B was greater than that of diet A and was associated with the added gallotannin.

TABLE 1

The mean content of several constituents in the experimental diets

Constituent	Variation in composition of the diets		
	A	B	C
	(%)	*(%)*	*(%)*
Tannin	1.76	4.27	4.06
Crude protein	22.10	21.24	13.05
Lignin[a]	11.03	10.48 (12.87)	13.97 (25.47)

[a] The values shown in parentheses are the "apparent lignin" values obtained prior to correction for tannin and for protein.

Qualitative tests for tannin indicated that the tannin extracted from alfalfa and the powdered tannin added to alfalfa to form diet B were of the same class but differed from that extracted from sericea. A water-soluble extract from the sericea hay changed to a greenish color on the addition of ferric ammonium sulfate, whereas that of the alfalfa hay and a solution of the tannin added to alfalfa turned blue. Also, a brick-red precipitate was obtained on boiling the aqueous extract of sericea in dilute mineral acids. On the other hand, there was no detectable change when dilute mineral acids were added to the aqueous extract of alfalfa or to a solution of the tannic acid powder and heated to boiling.

These qualitative tests suggest that the condensed tannin in sericea was of the catechol type, whereas the hydrolyzable tannin added in diet B and the hydrolyzable tannin in alfalfa were of the gallotannin type *(8)*.

Palatability trial. Intakes of feed and of water and gains in body weight by dairy calves when fed diets A and B were similar (Table 2). During the 10-day periods when the calves were fed diets A or B, however, the intakes of feed and of water and the gains in body weight were significantly greater than when they were fed diet C.

TABLE 2

Relationship between recoverable tannin content of diets[a] fed and the consumption of dry forage and of water and the change in body weight of young dairy animals during 10-day periods

	Mean response by diets			L.S.D.[b]
	A	B	C	P = 0.05
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*
Dry forage consumed	67.3	72.9	30.4	13.8
Water consumption	333.2	335.7	239.0	7.2
Change in body weight	11.7	11.0	−10.8	13.5

[a] Recoverable tannin content by diets: A, 1.76; B, 4.27; C, 4.06.
[b] L.S.D. is least significant difference.

Digestibility trial. The powdered gallotannin added to alfalfa hay (diet B) did not have a significant effect on the apparent digestibility of dry matter or of crude protein (Table 3). However, the apparent digestibility of the dry matter of diet B was higher than that of diet C in the amount of 28.4%. Also, the coefficient of apparent digestibility of crude protein for diet B was significantly greater than that of diet C. The apparent digestibility of crude protein in diet B was 35.3% higher than that of diet C.

TABLE 3

Mean digestibility of dry matter and of crude protein of test diets[a]

Diets	Dry matter	Crude protein
	(%)	*(%)*
A	72.3	78.7
B	73.5	79.3
C	45.1	44.0
Least significant difference	3.4	2.2

[a] Mean of three steers.

DISCUSSION

The concentrations of tannin in the sericea and the alfalfa fed in this study were similar to mean values reported for these forages by Olson and Elrod *(19)*. However, Herman *et al. (11)* have reported concentrations of tannin in alfalfa that were ten times as high as that in diet A. Apparently there is a wide range in the tannin concentration of sericea *(6, 19, 20, 23)*. It has been shown that sericea leaves contain more tannin than sericea stems *(5)*. Some reports do not indicate whether or not the tannin concentration shown represents the entire plant or the leaves only. Therefore, a comparison of all reports on tannin con-

centrations in forages may not be meaningful. Furthermore, variance in reports of different investigators may result from the method by which tannin was determined. According to Wilkins *et al. (23)*, the formaldehyde precipitate method of determining tannin gives values approximately 25% higher than the official method. Herman *et al. (11)* employed the hide powder method of the American Leather Chemists Association *(3)*. Olson and Elrod *(19)* did not specify the method used to determine tannin. The different methods used in the analyses may explain the marked differences in the values reported.

The lignin content of sericea was higher than that of alfalfa. In view of the coarse wiry stems of sericea, this would be expected. The apparent lignin content of sericea was reduced approximately 45% by extraction of tannin and correction for crude protein. Most of the reduction was associated with correction for crude protein, but a marked reduction in apparent lignin was observed when tannin was extracted from diets B and C prior to determining lignin.

The results of this study and those of several aforementioned investigations show that sericea is relatively low in palatability and digestibility and that the productive performance of animals fed this legume is low. The sericea fed in this study contained more tannin, but of a different class than that of the alfalfa hay fed. The relatively high tannin content of sericea has been associated with the relatively low consumption of this forage by farm animals. It is possible that different classes of tannins have varied effects on palatability and on digestibility, but in view of the many characteristics common to all tannins this would not be expected. Both gallo and catechol type tannins have an astringent taste and precipitate proteins by formation of insoluble tannates *(8)*. It would appear, therefore, that any effect of tannin on the quantity of forage consumed by livestock would be due to the astringent taste and that any effect on digestibility would be associated with the property of tannins which causes precipitation of proteins. Thus, for the relationships evaluated in this study, it seems that the tannin in alfalfa and the gallotannin added to alfalfa may be considered to possess the same qualities as the condensed tannin in sericea. Since the levels of recoverable tannin in diets B and C were approximately the same, apparently tannin does not account for the low level of consumption of diet C. This does not preclude the possibility that under cafeteria conditions, dairy cattle would prefer lines of sericea containing low levels to those containing high levels of these astringent-tasting compounds. Probably the acceptability of sericea by livestock is influenced by the coarseness of the plant, which increases with maturity. However, this would not apply to the ground sericea fed in this study.

The water intakes of the calves when fed diets A and B were similar but significantly greater than the intake when they were fed diet C (Table 2). This indicates that the tannin content of the diet did not affect the water intake of the calves. It is possible that the quantity of water consumed by the calves was influenced by the amount of dry matter ingested. The lower intake of water when sericea was the sole roughage fed may explain in part the relatively low milk production obtained in other studies *(9, 10, 11, 13, 14, 24)*.

The results of the digestibility studies suggest that the unbound tannin of

sericea does not account for the low coefficients of apparent digestibility of dry matter and of crude protein (Table 3) of this legume. Hence, if tannin has any effect on the digestibility of crude protein in sericea it would appear that it occurs in one or more of the following ways: *(a)* some of the naturally occurring tannin in the lespedeza sericea plant is combined with some of the protein in that plant as an indigestible protein-tannate; *(b)* tannin in lespedeza sericea is released in such a manner that it combines with the protein; or *(c)* tannic acid powder in diet B may have been inactivated in the upper part of the gastro-intestinal tract before the alfalfa-protein was in solution. Also, it is possible that the differences in the types of tannin involved in this study explain the lack of a detectable effect on digestibility of diet B, but, for reasons pointed out previously, this would not be expected.

The low digestibility of dry matter in the sericea probably was associated with the high lignin content of this forage, which averaged 13.97% as compared to 11.0% for the alfalfa hay. In addition, the relatively low coefficient of digestibility of the crude protein of sericea results in a lower digestible dry matter content of the forage.

The lower levels of consumption of feed and water by the animals on diet C, compared to those of animals on diets A and B, probably explain the loss in body weight.

SUMMARY

Sericea contained more tannin and lignin and less crude protein than alfalfa hay. The tannins in these two hays were of a different class. Powdered gallotannin added to alfalfa hay did not affect significantly the consumption of dry matter and water or the gains in body weight of 6-month-old dairy calves. Also, the apparent digestibility of dry matter and of crude protein in alfalfa hay was not affected significantly by the tannin content of the diets. The young dairy animals consumed more of the alfalfa hay with gallotannin added than of the sericea hay, which contained a similar quantity of a different class of tannin. In addition, the digestibility of dry matter and of crude protein of sericea was less than that of alfalfa hay with gallotannin added.

The results of this study suggest that the lignin content and the low coefficient of apparent digestibility of crude protein in lespedeza sericea account for the relatively low digestibility of the dry matter of this forage.

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THE PENETRATION OF LIGHT INTO MILK[1]

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It has long been known that light causes changes in milk. However, there is no reliable information on how far light penetrates into milk. To secure such information, the amount of light transmitted by layers of milk of increasing thickness was determined. Care was taken to measure all of the light transmitted, both the scattered and undeviated rays, since both are capable of producing chemical changes.

APPARATUS AND PROCEDURE

A schematic diagram of the light transmission apparatus is shown in Figure 1. The apparatus consisted essentially of a hollow sphere coated on the inside with magnesium oxide, a multiplier photometer, and an absorption cell. Virtually all of the light transmitted by the milk was trapped in the sphere, and its

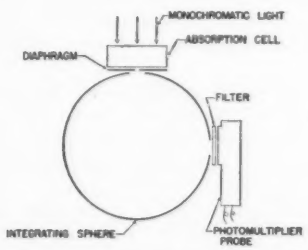

FIG. 1. A schematic diagram of the apparatus designed to measure both the scattered and undeviated rays transmitted by milk.

intensity was measured by the photometer. The photometer (model 520M, Photovolt Corp., New York) was equipped with a 28-B photomultiplier tube. Monochromatic light of high purity was produced by passing the rays from a General Electric AH-4 mercury arc through a Bausch and Lomb 250-mm. concave grating monochrometer. The power input to the arc was held constant with a voltage regulator.

The absorption cell consisted of two parallel glass disks (Corning Vycor 7910), separated by a ring. By using rings of different thickness it was possible to vary the depth of the milk layer from 0.025 mm. to 19.70 mm. The transmission of the absorption cell containing a layer of water less than 0.025 mm. thick was taken as 100%.

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[1] Some of the data appearing in this paper were taken from a thesis presented by the senior author to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, February, 1954.

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Transmission data were obtained for commercial samples of skim, whole, and homogenized milk at the following wave lengths: 5780, 5460, 4360, 4050, 3650, 3340, 3130, and 2540 Å. When making measurements at 4050 Å or less, a filter was placed between the sphere and the photometer to minimize the effect of fluorescent light. At 4050, 3340, and 3130 Å, Corning filter 7-54 was employed. This filter has a peak transmission at 3200 Å and cuts off at 2300 and 4200 Å. At 3650 Å, Corning filter 7-39 was used. It has a peak transmission at 3650 Å and cuts off at 3100 and 4100 Å.

When milk was exposed to 2540 Å rays, it produced a fluorescence, much of which consisted of wave lengths less than 4100 Å. Hence, an ultraviolet transmission filter could not be used for excluding fluorescence. Instead, the unfiltered transmitted light was measured. This included the fluorescence plus the unabsorbed 2540 Å waves. Then the light transmitted by the milk and a Pyrex glass filter was measured. This included only the fluorescence, because Pyrex glass cuts off at 2800 Å. The transmission of the 2540 Å rays was estimated by subtracting the reading obtained with the Pyrex filter from that obtained without the filter.

RESULTS AND DISCUSSION

The transmission data are plotted on semilogarithmic graphs in Figures 2, 3, and 4. The data indicate that except in the far ultraviolet (2540 Å), light penetrates to an appreciable depth in milk. For example, an 18-mm. layer of whole milk transmitted more than 1% of the incident green (5460 Å) light. Even in the near ultraviolet (3650 Å), a 4.5-mm. layer transmitted 2% of the incident light. As might be expected, the skimmilk proved to be the most transparent, and homogenized milk, the least. The homogenized milk was considerably more opaque than whole milk, although both had approximately the same fat and solids content.

These transmission data are in marked contrast to those obtained by Schneck *(5)* and Supplee and Dorcas *(7)*. Their data, which are summarized in Table 1, suggest that milk is extremely opaque and that as a result photochemical reactions are confined to a thin surface layer less than 1 mm. in depth. Their low

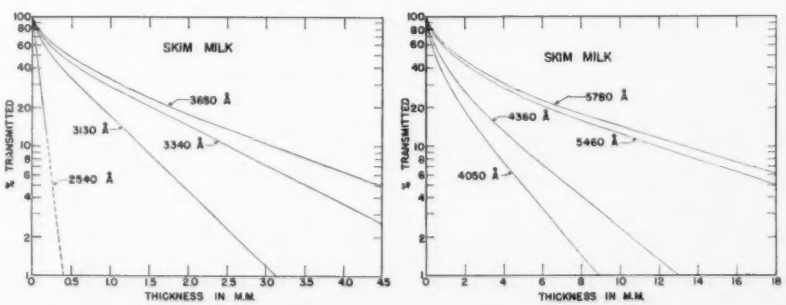

FIG. 2. Transmission data of a typical sample of skimmilk. Composition by the Mojonnier method: total solids 8.60%; fat 0.15%.

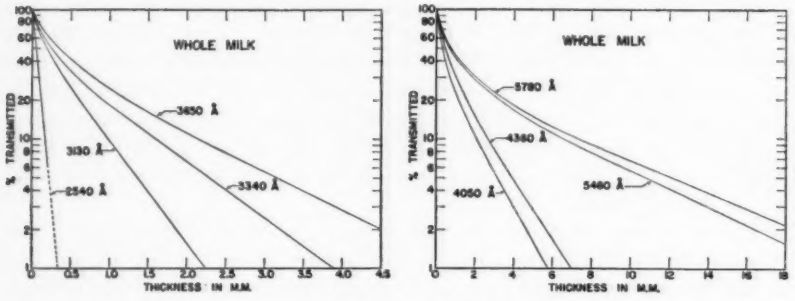

FIG. 3. Transmissison data of a typical sample of whole milk. Composition by the Mojonnier method: total solids 12.08%; fat 3.51%.

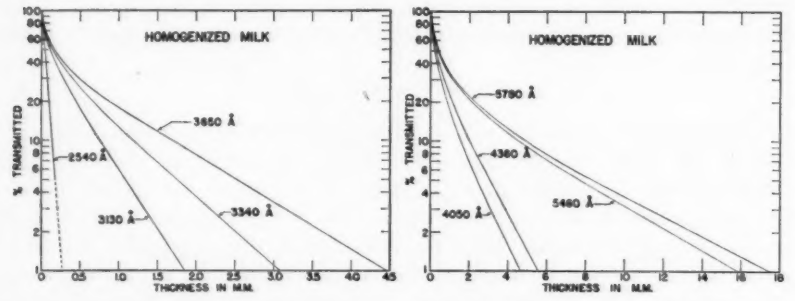

FIG. 4. Transmission data of typical sample of homogenized milk. Composition by the Mojonnier method: total solids 12.04%; fat 3.50%.

transmission values are due to the fact that their apparatus failed to measure much of the scattered light transmitted by the milk.

Except at 2540 Å, where the transmission data may be in error because of the short wave fluorescence, the data do not describe straight lines on the semi-

TABLE 1

Transmission data obtained for whole milk by Schneck (5) and by Supplee and Dorcas (7)

Author	Wave length	Thickness of layer	Transmission
	Å	*(mm.)*	*(%)*
Schneck	white light	0.1	13[a]
	white light	0.2	5.4
	white light	0.3	1.3
Supplee and Dorcas	4050	0.11	11[b]
	3650	0.11	11
	3130	0.11	7
	4050	0.23	8
	3650	0.23	8
	3130	0.23	3

[a] Fat content, 3.65%.
[b] Fat content, 3.6%.

logarithmic graphs. This means that Lambert's law, which is given by the equation

$$\text{Log } \frac{I}{I_o} = -kt$$

where I_o = the intensity of the incident monochromatic light
I = the intensity of the unscattered light transmitted by a layer of thickness t
k = the extinction coefficient,

cannot be used to predict the amount of light transmitted by milk. The inapplicability of Lambert's law is not surprising. In turbid solutions, $\frac{I}{I_o}$ does not represent the fraction of the incident light transmitted but that fraction of the incident light that is neither absorbed nor scattered. Nevertheless, Frahm *(3)*, cited by Burton *(2)*, employed Lambert's law to calculate the penetration of light into milk.

Assuming that Lambert's law did apply and using Frahm's extinction coefficients, the amount of light transmitted by a 1-mm. layer of milk was calculated. These calculated values are compared in Table 2 with the experimental values which include scattered light. Depending on the wave length, the experimental

TABLE 2

A comparison of the transmission data obtained by the application of Lambert's law with those obtained experimentally

Wave length	Per cent transmission of a 1.0 mm. layer of whole milk	
	Lambert law[a]	Experimental[b]
Å		
5780	1.8×10^{-5}	42.5
5460	9.1×10^{-6}	41
4360	6.7×10^{-7}	30
4050	2.2×10^{-7}	22
3650	5.4×10^{-8}	25
3340	1.3×10^{-8}	18.5
3130	4.7×10^{-9}	10.5

[a] Calculated using Frahm's extinction coefficients. Composition of Frahm's milk: fat 3.3%; protein 2.7%.
[b] Composition: fat 3.51%; total solids 12.08%.

values are from one million to one billion times larger than the calculated values. Possibly Frahm's Lambert law data accurately describe the transmission of unscattered light by milk. However, they give a misleading impression of the penetration of light into milk.

Although transmission data which include scattered rays provide a good idea of the penetration of light into milk, such data do not represent the actual illumination at various depths. Usually milk is exposed to light in glass bottles or pipes in which the milk may be considered to be of infinite depth. Hence, the information that would be of the most practical interest is the intensity of

light at depth t, not the intensity of light transmitted by a layer of thickness t. Two effects combine to make the amount of light at depth t greater than that transmitted by thickness t. First, when milk is enclosed in a cell for measurement, some of the light passing through the milk is not transmitted through the cell because of total and partial reflection at the exit glass-air interface. Second, when milk in bulk is exposed to light, particles at depth t are illuminated by light scattered back from deeper layers as well as by light coming from the surface.

A possible method for determining the intensity of light at various depths would be to measure the rate at which some light-sensitive substance was destroyed at these depths. An attempt along this line was made by using ascorbic acid as the light-sensitive substance. Ascorbic acid is ideally suited in that its rate of destruction in milk is directly proportional to the intensity of light and is independent of concentration *(1)*.

Whole milk, fortified with added ascorbic acid, was placed in a cylinder open at the top and fitted at the bottom with a piston. The milk was then set with rennet and exposed to May sunlight for one-half hour at noon. After the exposure, successive layers of the jelled milk, about 6-mm. thick, were forced from the cylinder by the piston and were analyzed for reduced ascorbic acid by the indophenol titration method of Sharp *(6)*. The results (Figure 5) show that measurable amounts of ascorbic acid were destroyed at depths approaching 26 mm.

Unfortunately the ascorbic acid data do not serve as an accurate measure of the relative amounts of light at various depths in milk. In the first place, the incident light was not monochromatic, and it is therefore impossible to relate the percentage of ascorbic acid destroyed to the light intensity. Attempts were made to use monochromatic rays from the AH-4 lamp, but they were not powerful enough to destroy ascorbic acid. In the second place, the action of the rennet increased the opacity of the milk. For example, at 5460 Å the rennet

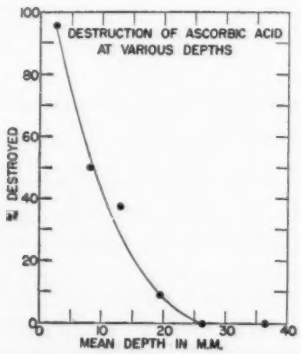

FIG. 5. The penetration of sunlight into rennet-clotted milk. The milk initially contained 30 mg. of reduced ascorbic acid per liter and was exposed for one-half hour to May sunlight.

caused the transmission of a 2-mm. layer of the milk to fall from 29 to 23%. Hence, the ascorbic acid data, like the transmission data, tend to underestimate the light intensity in milk. However, the data in Figure 5 provide additional proof that the effective depth of penetration is a matter of several centimeters rather than fractions of a millimeter.

Kon and Watson *(4)* exposed milk to June sunlight in a stack of three glass cells, each containing a 7-mm. layer. They found that all of the ascorbic acid in the top cell was destroyed in 15 minutes, but in 60 minutes none of the ascorbic acid in the bottom cell was affected. This implies that the sunlight failed to penetrate more than 14 mm. The apparent discrepancy between these results and those in Figure 5 is due to the two glass-air-glass interfaces, which prevented much of the sunlight from reaching the bottom cell.

SUMMARY AND CONCLUSIONS

Transmission data which included both the scattered and undeviated rays were obtained for typical samples of skim, whole, and homogenized milk at the following wave lengths: 5780, 5460, 4360, 4050, 3650, 3340, 3130, and 2540 Å. Contrary to reports appearing in the literature, the data indicated that light penetrates to an appreciable depth in milk. For example, at 5460 Å an 18-mm. layer of whole milk transmitted more than 1% of the incident light; at 3650 Å a 4.5-mm. layer transmitted 2% of the incident light.

Although transmission data which include scattered rays provide a good idea of the penetration of light into milk, such data tend to underestimate the intensity of light at the depths where the transmission is measured.

Lambert's law is of no value in predicting the total amount of light transmitted by milk or in estimating the depth of penetration of light into milk.

Sunlight is capable of destroying ascorbic acid in milk at depths approaching 26 mm.

ACKNOWLEDGMENTS

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THE DIFFUSE REFLECTION OF LIGHT BY MILK[1]

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Much of the light striking the surface of a layer of milk is reflected. The total reflection is made up of a specular reflection from the surface, which is independent of the thickness of layer, and a diffuse reflection from the interior, which depends upon the thickness of layer.

Diffuse reflection data are of value because they provide a basis for calculating the amount of light absorbed by milk and for estimating the effective depth of penetration of light into milk. Hence such data are presented in this paper.

APPARATUS AND PROCEDURE

Figure 1 shows the major components of the reflectance apparatus. They were: a hollow sphere coated on the inside with magnesium oxide, a reflectance cell, and a multiplier photometer. The incident monochromatic light traveled diametrically through the sphere and struck the reflectance cell. Practically

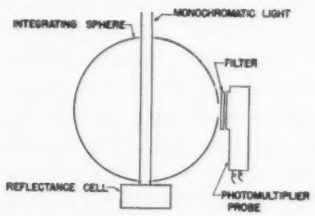

FIG. 1. A schematic diagram of the reflectance apparatus.

all of the light diffusely reflected by the milk particles was trapped in the sphere and its intensity was measured by the photometer. The reflectance cell consisted essentially of a hollow cylinder fitted at the top with a glass window (Corning Vycor 7910) and at the bottom with a black disk. By changing the distance between the window and the black disk, it was possible to vary the thickness of the milk layer in the cell from zero to 25 mm. An aluminum disk with a 1-mm. coating of magnesium oxide on its surface was used as the reflectance standard. The coating was prepared by allowing the smoke particles formed by burning reagent-grade magnesium turnings to deposit on the disk.

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[1] Based in part on material taken from a thesis presented by the senior author to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirement for the degree of Doctor of Philosophy, February, 1954.

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Reflectance data were obtained for commercial samples of skim, whole, and homogenized milk at the following wave lengths: 5780, 5460, 4360, 4050, 3650, 3340, 3130, and 2540 Å. When collecting data in the ultraviolet, filters were placed between the sphere and the photometer in an attempt to exclude fluorescent rays. Except at 2540 Å, the effect of fluorescence was small, most likely amounting to less than 4% of the light diffusely reflected. At 2540 Å the fluorescence appeared to be much stronger. It was impossible to make an exact evaluation of the strength of the fluorescence because the fluorescence was not monochromatic and the photometer was not uniformly sensitive to light of different wave lengths. The filters employed to exclude fluorescence were the same as those used in the transmission measurements *(2)*. Also, the same source of monochromatic light and the same photomultiplier were used.

RESULTS AND DISCUSSION

The reflectance data are given in Figures 2, 3, and 4. The data represent the percentage of light diffusely reflected, taking the light entering the milk layer as 100%. In order to obtain these data it was necessary to apply corrections for the light absorbed by the magnesium oxide of the reflectance standard, for

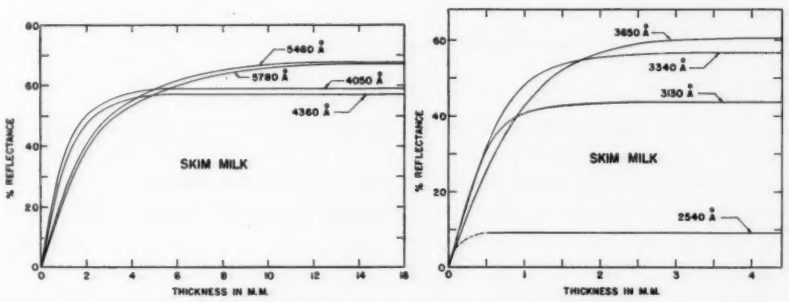

FIG. 2. Reflectance data of a typical sample of skimmilk. Composition by the Mojonnier method: total solids 9.03%; fat 0.59%.

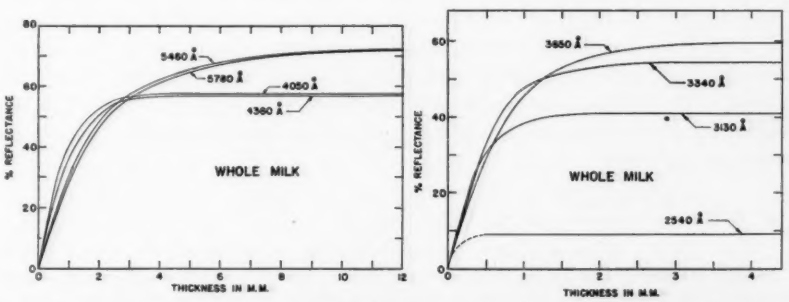

FIG. 3. Reflectance data of a typical sample of whole milk. Composition by the Mojonnier method: total solids 11.87%; fat 3.39%.

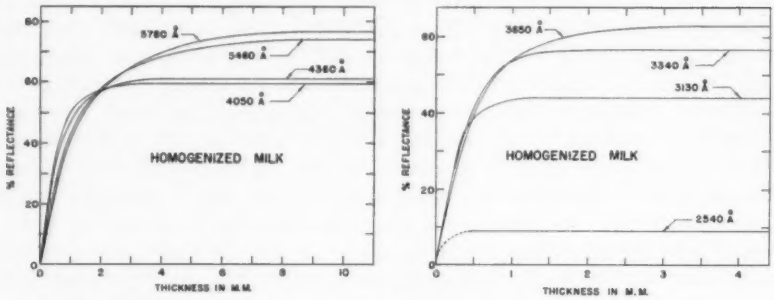

FIG. 4. Reflectance data of a typical sample of homogenized milk. Composition by the Mojonnier method: total solids 11.95%; fat 3.63%.

the light absorbed by the glass window of the reflectance cell, and for the light reflected from the surface of the glass window. If the incident light rays had been perfectly normal, it would not have been necessary to make the last correction. However, the incident rays were slightly divergent, so most of the light reflected from the surface of the glass window was trapped in the sphere and measured along with the diffusely reflected light.

The equations used in applying the corrections are derived below.

Let L = light incident on the window of the reflectance cell
C = light reflected from the surface of the window and trapped in the sphere
a = fraction of the light transmitted by the window
b = fraction of the light entering the milk layer which was diffusely reflected
f = fraction of the incident light reflected by the magnesium oxide standard
R = uncorrected reflectance—i.e., the reading given by the photometer

Neglecting the reflection at the glass-milk interface, the light entering the milk layer is $(L\text{-}C)a$. Assuming that the fraction of light transmitted by the window is the same for both the incident and reflected light,[3] the light that is diffusely reflected by the milk and travels back through the window is $(L\text{-}C)a^2b$. The light reflected by the magnesium oxide standard equals fL. Combining these terms gives us the equation:

$$R = \frac{(L-C)a^2b + C}{fL} \times 100 \qquad (1)$$

[3] Strictly speaking, this assumption is not justified, because on the average the reflected rays travel a greater distance through the glass than the incident rays. However, except at 2540 Å the absorbency of the glass is very low and hence the error introduced is in general not serious.

Since the photometer was set at 100 with the magnesium oxide standard, we can write:

$$fL = 100 \text{ or } L = \frac{100}{f}$$

Substituting $\frac{100}{f}$ for L and solving for b, we get

$$b = \frac{R - C}{\left(\frac{100}{f} - C\right) a^2} \qquad (2)$$

TABLE 1

Correction factors

Wave length	f[a]	a^2	C
(Å)			
5780	0.98	1.0	3.5
5460	0.98	1.0	3.5
4360	0.98	1.0	3.5
4050	0.97	1.0	3.5
3650	0.94	0.99	3.5
3340	0.93	0.98	3.5
3130	0.93	0.97	4.0
2540	0.95	0.73	4.0

[a] The f values at 5780, 5460, and 4360 Å were taken from Luckiesh *(3)*; the values at the remaining wave lengths were taken from Benford *et al. (1)*.

The values of f, a^2, and C used appear in Table 1. The values of f were taken from the literature. The values of a^2 were determined by measuring the transmission of the glass window and correcting for reflection losses. C was obtained by measuring the reflectance of a layer of milk of zero thickness, in which case b equals zero and equation 2 reduces to

$$R - C = 0 \text{ or } R = C$$

The reflectance data contain one error in addition to those for which corrections were made. When determining the reflectance, an area of about 6 sq. cm. on the surface of the milk was illuminated. Consider a small, but finite volume of milk near the center of the illuminated area. Most of the light diffusely reflected from this volume originally entered the volume from the glass-milk interface. However, some of the diffusely reflected light entered from neighboring volumes. This means that the volumes of milk at the boundary of the illuminated area reflect less light than those at the center because the volumes at the boundary did not receive their proper share of light from neighboring volumes. It is not known how serious this boundary error is, but it is hoped that the area illuminated was large enough to make it unimportant.

The reflectance data show that much of the light entering a layer of milk is diffusely reflected. For example over 70% of the green light (5460 Å) entering a 10-mm. layer of whole milk was diffusely reflected. In the near ultraviolet

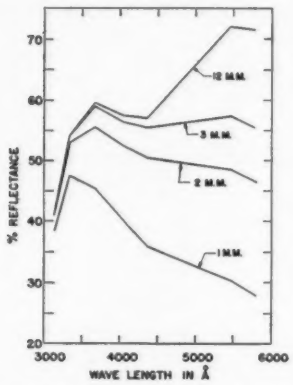

FIG. 5. The reflectance of whole milk as a function of wave length. The sample of milk is the same as that in Figure 3.

(3650 Å) over 60% was diffusely reflected. The homogenized milk reflected the most light, and skimmilk, the least. In Figure 5 the reflectance is plotted as a function of the wave length for layers of milk of a given thickness. The plot shows that thin layers of milk reflect a relatively greater percentage of light at the shorter wave lengths. This is a fact familiar to anyone who has observed that thin layers of milk have a bluish color.

Reflectance data, assuming they are accurate, can be used to estimate the amount of light absorbed by milk. As an example, consider a layer of whole milk which is at least 1 cm. thick and which is exposed to blue (4360 Å) light. Under these conditions the transmission loss is negligible and the diffuse reflection is 57%. Hence the fraction of the incident light absorbed is:

$$1 - 0.59 = 0.41$$

The value 0.59 was used instead of 0.57 to account for an assumed specular reflection of 4%. It should be remembered that light absorptions estimated in this way apply only to monochromatic light normal to the surface of the milk.

The graphs of the reflectance data provide information on the penetration of light into milk. In the case of a thin layer of milk, much of the incident light traveled through the layer and was absorbed by the black disk which served as the bottom of the reflectance cell, before it had a chance to be reflected. This accounts for the low reflectance of the thin layers. As the layer grew thicker, less light penetrated to the disk and more light had an opportunity to be reflected. Eventually a point was reached where such a small amount of light was absorbed by the disk that further increases in the thickness of the layer resulted in no measurable increase in the amount of light reflected. Twice the distance to this point was taken as the effective depth of penetration of the light. Twice the distance rather than the distance itself was used, because the light

which was reflected from the greatest depth must have traveled a distance of two thicknesses through the milk. The effective depth represents the thickness of the layer in which virtually all of the light absorption takes place.

TABLE 2

Effective depth of penetration of light into milk

Wave length	Effective depth		
	Skim	Whole	Homogenized
(Å)	*(mm.)*	*(mm.)*	*(mm.)*
5780	28	24	18
5460	26	24	18
4360	12	10	8
4050	11	9	7
3650	9	7	7
3340	7	6	5
3130	5	4	3
2540[a]	1	1	1

[a] The effective depth at 2540 Å may be in error, because the strong fluorescence made it difficult to determine at what thickness the reflectance reached its maximum value.

The values of the effective depth are presented in Table 2. The effective depths vary from about 2 cm. for yellow light to about 0.1 cm. for light in the far ultraviolet. The magnitude of the effective depths is about what one would expect on the basis of transmission data *(2)*.

SUMMARY AND CONCLUSIONS

Diffuse reflectance data were obtained for typical samples of skim, whole, and homogenized milk at the following wave lengths: 5780, 5460, 4360, 4050, 3650, 3340, 3130, and 2540 Å. The data showed that much of the light entering a layer of milk was diffusely reflected. The amount reflected varied from about 70% at 5780 Å to about 9% at 2534 Å.

A numerical example was given to show how reflectance data can be used to estimate the light energy absorbed by milk.

The effective depth of penetration of light into milk was estimated from the reflectance data. The values of the effective depth of penetration ranged from about 2 cm. at 5780 Å to 0.1 cm. at 2537 Å.

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THE MICROBIAL POPULATIONS OF THE GREEN PLANT AND OF THE CUT FORAGE PRIOR TO ENSILING

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Relatively little information is available on the microorganisms of green plants or of green forage used for silage either as to the numbers and species present or as to the effect that stage of maturity, environmental factors, such as weather, and methods of harvesting have on this flora. Although yellow pigmented bacteria normally predominate, it is obvious that the inherent microorganisms of the green plant include the lactic acid bacteria essential in the making of silage, as well as other species capable of causing spoilage under certain conditions. Various factors coincident with silage making through their effect on the microbial flora may aid or hinder desirable fermentation of the plant material in the silo.

Allen *et al. (1)* reported that under most conditions fresh grass contained millions of lactobacilli along with a predominance of coliform bacteria, some aerobic sporeformers, micrococci, streptococci, yeasts, actinomyces, and molds. Obligate anaerobes were detected in very small numbers. These investigators believed that the lactobacilli were strains of *Streptobacterium plantarum* that were identical to the lactobacilli in the ensiled grass. The coliform bacteria were identified as *Bacillus aerogenes grammis.* Stone *et al. (5)* obtained evidence that alfalfa and other green plants utilized for silage contain a plentiful supply of bacteria typical of the lactobacilli in silage. Clark *et al. (2)* found that the predominant yellow colony types on cotton plants were indistinguishable from *Xanthomonas malvacearum.*

In more recent reports, Thomas *(6)* and Stirling *(4)* indicate that the microorganisms on green forage plants consist of a heterogeneous microflora, which may vary considerably under different conditions, and that typical organisms which become active in silage fermentation are difficult to demonstrate because of their small numbers. They suggested that changes and fluctuations in the bacterial flora may affect the course of the fermentation of the silage.

This paper presents the results of preliminary studies on the numbers of microorganisms and some characteristics of the predominating kinds of bacteria that were found on fresh forage plants of different kinds and under different conditions and as affected by wilting and the harvesting process in preparation for ensiling.

PROCEDURE

Studies were made of the microflora on green plants utilized for silage and on other plants often mixed with the forage in the Beltsville area. Samples were taken to the laboratory and studied immediately.

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A 25-g. portion of finely cut plant material was placed with 475 ml. of physiological salt solution in a Waring blendor and agitated for 10 minutes at a slow speed obtained by setting the Powerstat variable transformer to deliver 40 v. on a 115-v. line. Preliminary studies with fresh green alfalfa showed that agitating the material for periods of 5 to 30 minutes gave only slight increases in bacterial counts. The 10-minute agitation at slow speed was chosen to avoid destruction of delicate organisms. Suitable dilutions were made, usually through one to a million, for the preparation of culture plates and other bacteriological studies.

Preliminary studies were made of the following media to evaluate their suitability in determining the numbers and kinds of bacteria on the plant material: tryptone glucose yeast extract agar consisting of 5 g. tryptone, 1 g. glucose, 3 g. yeast extract, and 15 g. agar per liter; Eugonagar (Baltimore Biological Laboratories); tomato juice agar consisting of 10 g. tryptone, 10 g. yeast extract, 200 ml. tomato juice, and 15 g. agar per liter; and nutritive caseinate agar (Difco Laboratories). The tryptone glucose yeast and tomato juice agar were adjusted to pH 7.0 before sterilization. The average bacterial counts obtained on each of the four media on 13 different samples of fresh alfalfa over a period of 6 weeks were 2,190,000; 1,908,000; 1,813,800; and 1,759,000, respectively. The intra-class correlation between the results on the different media was 0.96, which was highly significant. An analysis of variance of the bacterial counts showed that any difference in the value of the media was not significant. Nevertheless, tryptone glucose yeast extract agar appeared to be superior to the three other media for determining the numbers of bacteria on fresh plant material and generally at the time of ensiling.

Similar studies of the four media on wilted alfalfa and on alfalfa silage at different periods of storage showed that nutritive caseinate agar supported the largest number of bacteria from the wilted forage and during storage from 2 to 6 days, when coliform bacteria are likely to predominate. However, tomato juice agar supported the development of the largest number of bacteria from the silage after 13 days storage, was most favorable for lactic acid bacteria, and produced larger colonies on the culture plates. Therefore, tryptone glucose yeast extract agar and tomato juice agar were selected for routine use in the studies on green plant flora as well as on the forage material during the silage making process. Later studies included the use of Rogosa's medium *(3)* as a selective medium for lactobacilli. This medium was prepared by the formula given in the publication by using sorbitan monooleate in place of the author's intended ethylene oxide derivative (Tween 80). Recent studies comparing the two formulas of Rogosa's medium in plating fresh green forage and silage indicated that similar selective action was shown by both under the conditions studied.

RESULTS AND DISCUSSION

Numbers of microorganisms on fresh green plants. Studies of the microorganisms on fresh green plants showed the presence of a profusion of bacteria with considerable variation in numbers. Fresh green alfalfa contained a variable number of microorganisms in the same field during the same day. As shown in

Table 1, these variations did not correlate with the time of day. However, variations did not appear to be as great as increases in numbers with maturity of the plant.

TABLE 1

Numbers of microorganisms on green alfalfa in the morning and mid-afternoon at different intervals of the same season

Date	Time of day	Bacterial plate counts	Coliform bacteria	Molds and yeasts
		(000)		
5-9-51	9:15 A.M.	1,540	10^1	100
5-9-51	3:15 P.M.	6,240	10^1	400
5-16-51	9:30 A.M.	42,000	10^4	1,320
5-16-51	3:00 P.M.	28,400	10^2	560
6-1-51	9:30 A.M.	97,000	10^3	9,800
6-1-51	3:00 P.M.	99,200	10^2	9,000

TABLE 2

Numbers of microorganisms on the tassel or head and leaves of corn and orchard grass

Plant part	Date	Bacterial plate counts	Coliform bacteria	Molds and yeasts
		(000)		
Corn leaves	8-3-51	9,550	10^2	12,900,000
Corn tassel	8-3-51	455,000	$>10^7$	2,400,000
Orchard grass leaves	5-28-51	3,260	10^4	23,400
Orchard grass heads	5-28-51	520	10^3	1,000

Different numbers of microorganisms were found on different parts of the same plant at a given time, as indicated by examples in Table 2. Corn *(Zea mays)* tassel had very many more bacteria than did corn leaves. Corn tassel on several occasions yielded bacterial counts very much higher than corn leaves or parts of other plants studied. However, orchard grass heads were lower in counts of microorganisms than orchard grass leaves.

There were variations in the numbers of microorganisms on different kinds of plants. Table 3 gives examples of the number of microorganisms on seven kinds of plants during May and June, 1951.

TABLE 3

Numbers of microorganisms on different kinds of plants during May and June

Plant	Date	Bacterial plate counts	Coliform bacteria	Molds and yeasts
		(000)		
Horse tail	6-1-51	494	10^3	228
Oats	5-31-51	989	10^3	280
Orchard grass	5-11-51	340	$<10^1$	4,400
Thistle	6-19-51	2,600	10^3	1,000
White clover	6-15-51	11,780	10^1	13,200
Wild mustard	5-15-51	21,400	10^3	3,652
Wild onion	5-14-51	120	$<10^1$	500

TABLE 4

Numbers of microorganisms with advance in growth period or increase in maturity of plant

Plant	Date	Number of samples	Bacterial plate counts	Coliform bacteria	Molds and yeasts
			(000)		
Alfalfa	4/17-30/51	5	1,804[a]	10^{3}[a]	580[a]
Alfalfa	5/4-24/51	7	26,383[a]	10^{3}[a]	763[a]
Alfalfa	6/1-27/51	5	62,116[a]	10^{3}[a]	14,560[a]
Oats	6-2-53	1	2,600	10^{4}	1,000
Oats	6-9-53	1	3,350	10^{4}	15,300
Oats	6-29-53	1	23,100	10^{4}	26,000
Sour dock	5-25-51	1	28	$<10^{1}$	100
Sour dock	5-29-51	1	1,020	$<10^{1}$	2,848
Sour dock	6-14-51	1	6,960	10^{3}	680
Sour dock	6-19-51	1	4,840	10^{3}	320

[a] Average of samples studied.

Although there were many variations in the microbial populations on green plants, there seemed to be a tendency for the microorganisms to increase with the maturity of the plants and advancement of the season, as illustrated by three examples in Table 4. The samples of alfalfa were collected at the start of the growing season, at about the midpoint, and towards the end of the growing period before the first cutting.

The highest counts of molds and yeasts on forages were obtained during July, August, and September on alfalfa, corn, orchard grass, and soybeans, as shown in Table 5. The bacterial counts were high also on these samples.

The predominating kind of bacteria on fresh green plants. The predominating microorganisms of all green plants so far studied appear to be aerobic, chromogenic, nonsporeforming rod forms of bacteria. During the spring almost all the colonies of bacteria on the plates were yellow and appeared to be very much alike. Later in the season the color of colonies consisted of many more variations in the shades of yellow pigment; pink, buff, and nonpigmented colonies were usually well represented. Microscopic examinations of these chromogenic bacteria also showed much more diversity later in the season. It is believed that the predominating chromogenic bacteria are an indigenous plant flora. Soil samples from the same area as the plants gave an entirely different type of bacterial flora, as determined by the appearance of their colonies.

A preliminary study of 105 cultures from alfalfa, orchard grass, and soy-

TABLE 5

Summer forages with high counts of molds and yeasts

Plant	Date	Molds and yeasts	Bacterial plate counts	Coliform bacteria
			(000)	
Alfalfa	9-11-51	1,365,000	255,000	10^{5}
Corn leaves	8-3-51	12,900,000	9,550	10^{2}
Corn tassel	8-22-52	13,350,000	540,000	10^{6}
Orchard grass	7-5-51	1,700,000	172,500	10^{4}
Soy beans	8-29-51	1,945,000	249,000	10^{5}

beans revealed that most of the bacteria were chromogenic catalase-positive rods which yielded ammonia from arginine and liquefied gelatin. They showed variation in reducing nitrates and did not produce hydrogen sulfide. On carbohydrate media most of them produced acid slowly but were active in the hydrolysis of starch. A few of the chromogenic bacteria were analyzed for carotene, but none was demonstrated. When the yellow pigment-producing bacteria were inoculated into sterilized alfalfa, they, unlike some bacteria isolated from silage, did not change the pH value of the forage, and they were not recovered when the material was cultured at the time the pH was determined. In silages which showed a rapid lowering of pH, the chromogenic bacteria disappeared rapidly and were recovered only during the first few days after ensiling. It is probable that the chromogenic bacteria of the green plant, which constitute a large part of the initial microorganisms at the time of ensiling, may contribute to the exhaustion of oxygen in the freshly ensiled forage.

Attempts to demonstrate the presence of *Lactobacillus plantarum* and other similar organisms on fresh green plant material have been largely unsuccessful. Rogosa's medium, selective for lactobacilli, usually did not support the growth of bacterial colonies even in low dilutions of fresh green plant material. However, 44 bacterial cultures originating from fresh green plants have been isolated from colonies on Rogosa's medium. In general, they were short rod forms and cocci. They were Gram-positive nonpigmented bacteria capable of fermentating a number of carbohydrates. None of these cultures liquefied gelatin, hydrolyzed starch, or produced hydrogen sulfide. Most did not produce catalase, reduce nitrate, or curdle milk. Most formed ammonia in arginine broth and were active in the fermentation of glucose and sucrose, and a few utilized pentose sugars. They were similar to some kinds of bacteria active in the silage but none belonged to the groups of active acid-producing lactobacilli typical of the silage flora.

British workers *(1)* in their determination of coliform bacteria incubated the tubes at 30° and 37° C. and obtained many more bacteria at the lower temperature. The results in this study suggest that the coliform bacteria on plants in this area were favored by the lower temperature only slightly. Preliminary studies of a few of the coliform bacteria indicate that the majority of them are methyl red and indole negative and Voges-Proskauer and citrate positive. Since they liquefy gelatin, they have been identified as *Aerobacter cloacae*. The exact role of coliform bacteria in silage fermentation is not known, but in silages in which the pH value decreased rapidly they disappeared within a few days. In silages of slower acid development the coliform bacteria continued to be present for several weeks.

The numbers of microorganisms on alfalfa during preparation for ensiling. The microorganisms on green forage increased in numbers greatly during the period between cutting and ensiling, particularly when the forage was left to wilt in the field for two or more hours before it was chopped, loaded, and transferred to the silo. The data in Table 6 show there was approximately a 7 to 17-fold increase in microorganisms on alfalfa that had been wilted in the field for

TABLE 6

Increase in numbers of microorganisms on alfalfa during wilting

Date	Time of day	Condition	Bacterial plate counts	Coliform bacteria	Molds and yeasts
			(000)		
6-27-51	10:30 A.M.	As cut	1,815	10^3	3,750
6-27-51	1:05 P.M.	As blown into truck	12,550	10^4	31,500
7-15-51	4:00 P.M.	As cut	18,750	10^5	335,000
7-16-51	9:15 A.M.	As blown into truck	220,000	10^6	660,000
7-16-51	11:30 A.M.	As blown into silo	305,500	10^7	1,330,000
7-19-51	9:30 A.M.	As cut	75,500	10^6	445,000
7-19-51	2:30 P.M.	As blown into truck	550,000	10^6	700,000
7-19-51	3:20 P.M.	As blown into silo	575,000	10^6	1,315,000
8-20-51	10:30 A.M.	Cut with scythe	43,500	10^5	2,040,000
8-20-51	1:30 P.M.	Hand collected	87,500	10^5	1,540,000
9-21-51	10:40 A.M.	Cut with hand shears	184,000	10^5	1,340,000
9-21-51	2:20 P.M.	Hand collected	420,000	10^5	6,900,000

several hours previous to chopping and loading in the usual harvesting process. The results of two trials in which alfalfa was harvested by hand are shown at the bottom of Table 6. This material was finely chopped in the laboratory just prior to analysis. These two trials show that wilting alone may have accounted for about a twofold increase in numbers of microorganisms. The other data in Table 6 indicate that the normal field harvesting process consisting of chopping in the field, blowing onto the truck, and some delayed holding in the truck before the chopped material was blown into the silo may account for a substantial increase in the numbers of microorganisms. It is apparent that these increases in the numbers of microorganisms cannot be accounted for alone by concentration of the microorganisms as a result of the loss of 25% or less of moisture during the wilting. This increase did not appear to be due to increase of any particular kinds of organisms even though some typical silage bacteria were present and apparently increased, since they were not recovered from the fresh green plants. There was a substantial increase in molds and yeasts.

The rapid proliferation of microorganisms on fresh green plants attendant with the wilting and mincing of the plant material in preparation for ensiling suggests that a delicate equilibrium exists in the microorganisms-plant relationship. The added evidence that large populations of apparently indigenous bacteria are carried constantly by growing plants suggests a further significance to this microorganisms-plant relationship and the need for a much more thorough study in this field.

The increase in numbers of microorganisms during the wilting process may have considerable influence in rapidly exhausting the oxygen in the silo and may in part account for the effectiveness of this procedure.

SUMMARY

Large numbers of microorganisms were found on fresh green forage plants. The numbers of bacteria varied with the kind of plant, part of the plant, season,

and maturity of the plant. Increases in numbers of microorganisms occurred with increase in maturity of the plant. A definite increase in numbers of microorganisms was attendant with wilting and chopping of plant material in preparation for ensiling.

The predominating microorganisms on fresh green plants consisted of pigmented, aerobic, nonsporeforming, rod-shaped bacteria. Coliform bacteria also were found on fresh green plants in large numbers and increased during the harvesting process. A majority of the coliform bacteria studied belong to the genus *Aerobacter*.

Relatively few of the microorganisms isolated from fresh green plants were similar to bacteria present in silage, and none were typical of lactobacilli found in silage. These microorganisms found to be similar to the silage bacteria appeared to increase in chopped forage previous to ensiling.

The largest number of molds and yeasts on fresh green plants occurred during July, August, and September on alfalfa, corn, orchard grass, and soybean plants under conditions during which the numbers of bacteria also were high.

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MICROBIAL ACTIVITIES IN ALFALFA AND ORCHARD GRASS ENSILED UNDER CERTAIN CONDITIONS IN EXPERIMENTAL SILOS

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It is generally recognized that microorganisms are active in silage and that lactic acid bacteria perform a useful role in the preservation of the forage through the production of acids *(1, 2, 11, 14)*. The lactic acid bacteria obviously are on the forage at the time of ensiling *(1, 3)*, but they are present in such small numbers *(8)* that their usefulness in silage depends on favorable conditions for their growth and prolonged activity.

Control of bacterial activity in silage by the addition of mineral acids (the A.I.V. method) to arrest bacterial development *(16)* has been only partially successful *(6)* and not recommended by some because it is economically impractical. Similarly, the use of various additives in silage as selective inhibitors of destructive bacterial action is opposed and advocated by various investigators, and their usefulness generally must depend on basic studies under various conditions.

The effect of the addition of carbohydrates in various forms, such as molasses and milk by-products, on the bacterial flora of silage has been studied by various investigators *(2, 7, 18)*. Carbohydrates were found to be effective in lowering the pH of silage, but opinions differ as to their value in making high quality silage. Stone *et al. (14)* found that the addition of carbohydrates was indicated when the sugar content of the forage was low, but they believed that typical lactic acid fermentation took place irrespective of treatment and that after 2 days storage lactobacilli predominated. Salisbury *et al. (11)* found that the fermentation capacity was greater for simple sugars than for more complex carbohydrates, such as occur in cereal grains.

Investigators who have made detailed studies of ensiled forage have observed rapid changes in the microorganisms on the forage soon after ensiling *(5, 13, 15)* and have attributed these changes directly or indirectly to the presence of plant juices and adequate moisture. Results of recent studies *(8)* indicate that the change in microorganisms on the forage begins at the time the forage is cut.

Wilting of legume forages in the field to lower the moisture content has been recommended *(18)* and practiced widely in the making of silages, but the advantages of this method in the bacterial fermentation processes of silage making are largely unknown.

In general, the literature indicates there are various methods for enhancing lactic fermentation in silage, but very little fundamental information is available on practical methods of controlling the various fermentation processes in silage

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to prevent loss of nutrients. In view of the lack of adequate knowledge of silage microbiology and the increased emphasis placed on the utilization of forage crop silage in the ration, investigations were undertaken to study microbial activities in forages stored in experimental silos and especially as affected by kind of forage, wilting, sealing of the silo, and adding of SO_2 as a preservative.

GENERAL EXPERIMENTAL METHODS

Sampling, chemical and bacteriological procedures. Samples of silages were collected in a hollow tube by boring into the silage through "portholes" in the silos. The method is illustrated in Figure 1. About a 1,000-g. sample of silage

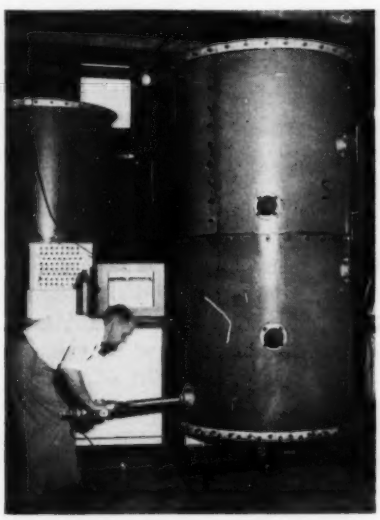

FIG. 1. A sample of silage was obtained by boring into the silage through a "porthole" of a glass-coated steel silo.

was well mixed in a large, clean can with hands covered with sterilized gloves. A portion of the sample was placed in a refrigerator for a short time after sampling until preparations were made for microbiological studies. Another portion was preserved for chemical analysis.

Determinations of pH values were made with glass and calomel electrodes in firm contact with the forage. Sugar determinations were made by use of Somogyi's sugar reagent *(12)* with clarification of extracts by resin treatment prior to the sugar analysis *(17)*. Ammoniacal and total nitrogen determinations were made by procedures listed in the *Manual of Methods (4)*.

The methods used in the determination of bacteria, yeasts, and molds in silages were essentially the same as those employed in the study of green plants *(8)*. Since there was particular interest in following the changes in the microorganisms from the indigenous forms on the forage to the lactic acid micro-

organisms developing in the silage, tryptone glucose yeast extract agar and tomato juice agar were selected for routine use in enumerating the numbers of bacteria in the silage. Representative colonies for pure culture study were isolated from the tomato juice agar routinely. In 1952 Rogosa's medium *(10)* was used also because of its selectivity for lactobacilli. The numbers of anaerobes in the silage were determined at certain intervals, but the methods and results will be discussed in another paper.

Experiment 1. A comparison of microbial activities in ensiled fresh orchard grass with and without sealing the silo, and in ensiled wilted orchard grass. Three 4 ft. × 8 ft. steel silos, as shown in Figure 1, were filled with chopped first-cutting orchard grass on May 6, 1952. Succulent orchard grass of 79.5% moisture was stored in two of the silos. Orchard grass of the same crop wilted to 66.5% moisture was stored in the third silo. The forage was tramped during filling, and on top of each of the forages were placed a layer of Sisal paper, an 18-in. layer of fresh chopped forage, another layer of paper, and then a steel follower and weights totaling 1,000 lb. to provide uniform pressure simulating a farm size silo. After the weights were in place, one silo with fresh orchard grass was sealed to exclude air, and the other two silos were left unsealed.

TABLE 1

Bacteriological and chemical data on orchard grass silages

Description	Age	Bacterial plate counts[a]	Sugar	pH value	Ammoniacal nitrogen[b]	Crude protein	Dry matter
	(days)	*(000)*	*(%)*		*(%)*	*(%)*	*(%)*
Fresh	0	2,445	9.32	5.84	0.28	21.33	20.10
orchard	2	1,335,000	5.68	4.38	0.44	21.30	20.92
grass—	5	1,920,000	4.46	3.95	0.62	20.84	21.16
silo not	9	865,000	0.57	3.95	0.77	21.07	20.17
sealed	16	211,500	0.85	3.85	0.91	21.84	19.71
	41	60,000	0.04	3.89	—	—	20.63
	75	4,250	0.40	3.75	2.46	20.55	17.80
	140	—	0.05	4.41	2.14	22.60	20.25
	177	145,000	—	—	—	—	—
Fresh	0	2,445	9.32	5.84	0.28	21.33	20.10
orchard	2	1,930,000	4.75	4.58	0.48	21.38	21.33
grass—	5	2,065,000	—	4.00	0.66	21.84	20.50
silo	9	850,000	0.57	3.93	0.86	21.91	19.50
sealed	16	370,000	0.62	3.85	1.01	21.15	19.16
	41	104,000	0.28	3.88	1.24	21.62	19.88
	75	3,100	0.21	3.85	1.41	18.31	18.92
	140	—	0.09	4.06	1.65	20.36	21.00
	177	176,000	—	—	—	—	—
Wilted	0	14,250	10.47	5.77	0.17	17.72	33.45
orchard	2	134,500	8.66	5.90	0.30	17.66	32.86
grass—	5	1,240,000	2.53	5.20	0.71	18.10	32.00
silo not	9	2,765,000	3.11	4.52	1.05	18.76	31.75
sealed	16	1,695,000	2.63	4.12	1.29	18.85	31.81
	41	232,000	1.78	4.12	1.73	18.79	32.26
	75	11,900	1.61	4.04	1.86	16.13	31.17
	140	—	2.34	4.06	1.97	18.76	33.50
	177	3,870	—	—	—	—	—

[a] On tomato juice agar.
[b] Calculated as protein.

Bacteriological and chemical data on these silages, as shown in Table 1, indicate a slightly more rapid and early increase in activity of bacteria in the fresh orchard grass silage from the sealed silo than from the unsealed silo. Otherwise, there is a similarity in the results for these two silages. In contrast, the data for the wilted orchard grass show consistent differences.

The numbers of bacteria increased rapidly in the ensiled high moisture forage to reach a maximum in 5 days and thereafter decreased very rapidly. There was rapid acid production; and chromogenic bacteria, yeasts, and molds decreased rapidly. Coliform bacteria, after an increase at 2 days storage, also decreased very rapidly. However, in the wilted forage the numbers of bacteria increased less rapidly to reach a maximum in 9 days and persisted in large numbers for over 16 days before a marked decrease occurred. The acid production was retarded, and the numbers of coliform bacteria and yeasts increased in the ensiled forage and remained in large numbers beyond 16 days. At 177 days after ensiling, the numbers of bacteria in the high-moisture silages had increased beyond that present at 41 days, but those in the wilted forage silage continued to decrease.

Bacterial activity also occurred more rapidly in the ensiled high-moisture forages, as indicated by the rate of sugar utilization and the rate of acid production. In these silages over half of the sugars had been utilized and the pH value was decreased to 4.00, or below, 5 days after ensiling, which coincided with the peak of bacterial development. At 9 days practically all the sugar had been utilized, and the pH values remained low during the remainder of the storage period.

However, in the lower moisture silage the utilization of sugars occurred slowly at first, then proceeded more rapidly as the numbers of bacteria increased. At 5 days of storage over two-thirds of the sugars had been utilized, but the maximum number of bacteria and low pH value did not occur until 9 and 16 days, respectively, after ensiling. At 140 days of storage the residual sugar in this lower moisture silage was about 2%. The pH values tended to remain at a slightly higher level during most of the storage period. These data suggest that the sugars and other nutrients for bacterial growth were less readily available in the wilted or low-moisture forage.

Studies on the kinds of bacteria isolated from the tomato juice agar indicate a higher percentage of lactic acid-producing bacteria and their earlier activity in the high-moisture silages. These bacteria occurred later and persisted longer in the low-moisture silage. Groups of bacteria characterized by the production of ammonia from amino acids, such as arginine, were found to occur predominantly in all three silages after 41 days of storage, which may explain in part the increase of ammoniacal nitrogen during the latter part of the storage period. The difference in the percentages of crude protein between the silages made from fresh forage and from wilted forage has not been adequately explained.

There was evidence that sealing the one silo favored the development of some bacterial activity. At 2 days the silage in the sealed silo had a larger number of bacteria and less sugar than that in the unsealed silo, but the pH value was

higher. Additional data show that a greater number of bacteria occurred at 5, 9, 16, 41, and 75 days of storage, respectively, on Rogosa's medium, selective for lactobacilli, in the silage from the sealed silo than occurred in the high-moisture silage in the unsealed silo. A higher percentage of the cultures isolated from the tomato juice agar at 5, 9, 16, and 41 days were lactobacilli. Pediococci, which were isolated from the forage at the time of ensiling, were also found in high numbers earlier in the silage from the sealed silo.

Examination of the 200 to 300 lb. of fresh cut forage placed at the top of each silo above the paper showed definite advantages to sealing the silo. The top layer in the sealed silo was found to contain only 2 lb. of spoiled forage, and 88% of the dry matter was preserved. The top layer of forage in the unsealed silo contained 92.5 lb. of spoiled forage, and 44% of the dry matter was preserved. The top layer in the unsealed wilted-forage silo contained 96 lb. of spoiled forage, and 53.5% of the dry matter was preserved. These results indicate that the method of sealing was reasonably effective. However, the silage in the sealed silo below the paper showed 95.54% preservation of dry matter, as compared to 97.32% and 98.14% dry matter preserved, respectively, in ensiled fresh and wilted forages in the unsealed silos.

Experiment 2. A comparison of microbial activity of alfalfa silage as shown in ensiled fresh cut forage, in fresh cut forage treated with SO_2, and as affected by slight wilting. Three 4 ft. × 8 ft. steel silos, as shown in Figure 1, were filled with the first cutting chopped alfalfa on June 3, 1952. Fresh cut alfalfa of 77.6% moisture was stored in one silo. Fresh cut alfalfa of 77.9% moisture plus 0.25% SO_2 was stored in the second silo. The third silo was filled with alfalfa slightly wilted to 72.1% moisture. The forage in all three silos was well tramped,

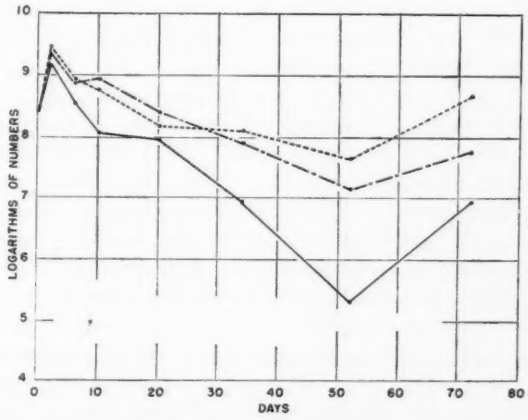

FIG. 2. Colony counts of bacteria on tomato juice agar from alfalfa silages.

Legend - - - - Fresh alfalfa (77.6% moisture).
— · — Wilted alfalfa (72.09% moisture).
——— Fresh alfalfa plus sulfur dioxide (77.94% moisture).

then covered with a layer of paper, a layer of fresh chopped forage, a steel follower, and 1,000 lb. of weight in the same manner as in the first experiment, but all three silos were left unsealed.

The trends of the bacterial populations, as determined from colony counts on tomato juice agar, in these silages are illustrated in Figure 2. The graph indicates definitely that the addition of SO_2 to the ensiled forage inhibited the over-all bacterial population. Similar effects were obtained in the previous year's study when SO_2 was added to second cutting wilted alfalfa of 58.7% moisture as it was ensiled in a 10 ft. × 25 ft. concrete silo. The plotted values also show that the numbers of bacteria in the ensiled slightly wilted forage followed a slightly more steady decrease than in the control. All three silages showed an increase in numbers of bacteria at 72 days of storage.

Tryptone glucose yeast extract agar gave definitely higher counts on the forages at the time of ensiling than did tomato juice agar. Rogosa's medium, selective for lactobacilli, gave low counts. After 2 days of storage, the tomato juice agar gave the higher counts, and Rogosa's medium supported the growth of a very large number of bacteria, indicating that a proportion of the bacteria in all three of the silages after 2 days storage was lactobacilli or closely related bacteria.

In all three silages chromogenic bacteria disappeared soon after ensiling. Coliform bacteria were not found in any of the three silages at the sixth day of storage. The two fresh-cut alfalfa silages did not contain yeasts and molds at 2 days of storage or thereafter, and the wilted alfalfa silage in only insignificant numbers. Soon after ensiling of the forages the predominating forage microorganisms in all three silages were replaced by nonpigmented bacteria, many of which probably belong in the genera *Lactobacillus* and *Pediococcus (9)*. These were replaced later by slow acid-producing ammonifying bacteria, most of which probably were heterofermentative lactobacilli, since they grew on Rogosa's medium selective for lactobacilli.

Bacterial activity in the three silages, as indicated by methods selective for lactobacilli and by chemical changes that occurred in the process, is shown in Table 2. The data indicate that the addition of SO_2 did not greatly inhibit the growth of bacteria during the first few days. As a result, the sugars were utilized rapidly and the pH value decreased to below 4.3. However, after this activity, the results indicate that the action of SO_2 rapidly reduced the numbers of bacteria in the silage as compared to the other silages, but not before a more selective acid-forming microflora and a lower pH value than occurred in the other silages had become established.

The effect of slightly wilting the alfalfa was not very apparent in the early storage period. The silages from wilted and fresh-cut alfalfa without SO_2 were similar in several respects. In both, the sugars were almost completely utilized in 2 days. Both showed a gradual increase in ammoniacal nitrogen. However, the silage from the slightly wilted forage appeared to show a more controlled fermentation process, as indicated by the more gradual diminution of the numbers of bacteria and a more constant, although higher, pH value. Apparently the

TABLE 2

Bacteriological and chemical data on alfalfa silages

Description	Age	Bacterial plate counts[a]	Sugar	pH value	Ammoniacal nitrogen[b]	Crude protein	Dry matter
	(days)	*(000)*	*(%)*		*(%)*	*(%)*	*(%)*
Control alfalfa	0	15	4.57	5.83	0.21	20.13	22.40
	2	300,000	0.29	4.52	1.04	20.01	23.87
	6	580,000	0.06	4.40	1.09	18.47	25.31
	10	186,500	0.06	4.20	1.48	19.89	26.69
	20	101,500	0.12	4.38	1.82	19.00	24.33
	34	63,500	—	4.15	1.55	19.70	24.67
	52	112,000	0.18	4.12	2.13	19.10	25.00
	72	450,000	0.14	4.32	2.09	20.35	23.67
	127	——	0.08	4.70	2.46	19.01	24.50
	195	29,500	0.15	4.57	2.56	20.31	23.50
SO_2-treated alfalfa	0	15	4.51	5.79	0.22	20.37	22.06
	2	1,480,000	0.98	4.50	0.96	20.34	23.27
	6	164,500	0.48	4.32	1.07	20.20	24.31
	10	71,500	0.29	4.15	1.05	19.50	23.00
	20	15,500	0.83	4.32	1.53	20.43	23.41
	34	2,550	0.56	4.13	1.60	19.47	23.83
	52	10	0.24	4.09	1.59	20.59	24.00
	72	14,400	1.08	4.04	1.72	19.64	24.00
	127	——	2.39	4.15	1.75	19.84	25.50
	195	715	1.17	4.11	1.79	20.32	24.60
Wilted alfalfa	0	20	4.27	5.71	0.26	19.38	27.91
	2	300,000	0.08	5.05	1.62	21.55	28.52
	6	835,000	0.04	4.80	0.65	19.13	29.00
	10	720,000	0.06	4.45	2.56	19.65	28.27
	20	152,000	0.17	4.52	2.81	20.57	27.75
	34	73,000	0.19	4.51	2.85	20.15	27.17
	52	13,150	0.02	4.31	2.95	19.70	27.75
	72	42,500	0.22	4.34	2.81	19.67	28.83
	127	——	0.04	4.40	3.04	20.98	27.50
	195	81,500	—	4.43	2.98	20.71	26.75

[a] On Rogosa's medium.

[b] Calculated as protein nitrogen.

exhaustion of the available sugars was closely associated with other limitations for bacterial growth, and the fermentation process gradually slowed to a minimum.

On the other hand, the silage from the fresh-cut alfalfa showed a more erratic microbial activity. After the pH value decreased to 4.2, it rose temporarily to 4.38 at 20 days, then decreased to 4.12, but rose again and remained at a relatively high level after 72 days of storage. It appears that distinct changes in bacterial flora occurred. The colony appearance of the bacteria was observed to be different at the 20th and 34th days of storage, and the increase in numbers of bacteria at the 52nd and 72nd days of storage indicates that a secondary type of fermentation occurred which may have caused the increase of the pH value. Studies of bacterial cultures isolated from the silage also indicate that a significant part of the flora at 34 days of storage and thereafter consisted of slow acid-producing atypical pediococci which usually have not been found in lower moisture silage. The data on the silage made from high-moisture alfalfa indicate that under these conditions of ensiling, in which there developed a scarcity of available sugar soon

after ensiling, other less fastidious bacteria developed in the silage after the 34th day of storage in place of the rapid acid-producing lactobacilli, which may have accounted for the rise in pH value and some deterioration of the silage.

Data for these silages show that the percentages of dry matter preserved below the division papers were 90.2 in the control high-moisture alfalfa, 93.3 in the slightly wilted alfalfa, and 95.3 in the SO_2-treated high-moisture alfalfa. The control alfalfa provided the least preservation of stored dry matter, and it may be assumed that a large part of the loss was due to fermentation losses, which continued as long as bacterial nutrients other than sugars were available. It is probable that the presence of greater quantities of available sugars may have resulted in a low pH value, thereby inhibiting further degradation due to microbial activity in the control silage.

DISCUSSION

The data on the orchard grass silage experiment indicate that a more rapid increase in numbers of bacteria, shorter persistence of miscellaneous microorganisms, earlier predominance of lactobacilli, earlier production of acid, and more rapid utilization of sugar occurred in ensiled fresh orchard grass of 79.5% moisture than in the same forage wilted to 66.5% moisture. Sealing one silo containing fresh forage further accentuated the microbial activity and lowering of the pH value and provided greater preservation of the forage at the top of the silo.

The wilted forage silage, because of its high dry matter content and the higher percentage of preserved dry matter, was more desirable silage from the standpoint of animal feed. Greater microbial activity, as occurred in the high-moisture forage, apparently was not essential or even desirable. On the other hand, rapid acid production and low pH values in silage have been shown repeatedly *(2, 6, 14)* to be essential for proper preservation of ensiled forage. It is probable that, under the conditions of ensiled wilted forage in which there was a continual residue of sugar and a relatively low pH value after 9 days of storage, undesirable fermentation was inhibited even though acid production was retarded. The results indicate that a high sugar-containing forage may be ensiled satisfactorily from a biological standpoint either as fresh or wilted forage.

The experiment on alfalfa silages showed that the addition of 0.25% of SO_2 to fresh chopped alfalfa of 77.9% moisture permitted rapid increases of lactobacilli and other similar bacteria to a maximum number at 2 days of storage. It also permitted rapid acid production, which resulted in a low pH value that remained fairly constant throughout the storage period. It greatly inhibited other bacterial activity after 6 days, thereby preserving a higher percentage of dry matter than occurred in the silage of the untreated control or in the wilted forage of 72.1% moisture.

The control silage containing 77.6% moisture showed erratic microbial activities as to numbers and kinds of bacteria and pH values. These changes in pH values indicate the unreliability of a single pH determination at the end of the

storage period as a measure of acid production. The erratic microbial activities also resulted in less preserved forage and a higher production of ammoniacal nitrogen. The silage made from the slightly wilted forage showed controlled microbial activity, which in spite of the higher pH value gradually slowed to a minimum with a higher percentage of preserved dry matter than in the control. These results indicate that alfalfa of less than 5% sugar can be ensiled more satisfactorily when wilted or by the addition of SO_2 to the fresh forage. It appears desirable to have a proper balance between the moisture and sugar content of the forage, preferably a relatively low moisture content with limited amounts of sugar to provide early acid production, thereby producing a low pH value and decreased microbial activity.

SUMMARY

The bacterial flora of the forage at the time of ensiling consisted largely of chromogenic and coliform bacteria. Various lactic acid bacteria and related species were found in only small numbers. The numbers of bacteria increased rapidly the first 2-9 days after ensiling. The rate of increase was faster in the fresh cut high-moisture forage and slower and of longer duration in the wilted or lower-moisture forage. After the rapid increase to a high count, the numbers of bacteria decreased rapidly or gradually depending upon high moisture and rapid acid production or lower moisture, as with wilted forage with slow acid production. The predominating forage microorganisms disappeared during the first few days, and lactic acid-producing bacteria became predominant. A later increase in numbers of bacteria occurred in most silages, which appeared to be a secondary fermentation.

The lactic acid bacteria and acid production were favored by chopped high-moisture forage, by an abundance of available sugar, and by storage of the forage in a sealed gas-tight silo.

Fresh-cut forage of high sugar content appeared to provide the inherent means of naturally controlled fermentation. Alfalfa low in sugar lacked the capacity for adequate acid production, and its preservation as silage was benefited by addition of SO_2 and by wilting and probably would have been benefited also by the addition of some form of readily fermentable carbohydrate.

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THE CAVITATION THEORY OF HOMOGENIZATION

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Several theories have been proposed to explain the subdivision of fat globules that occurs during homogenization. One of these is cavitation. According to this theory, the fat globules are shattered as the result of the collapse of vapor bubbles formed in regions of high velocity and low pressure.

In order to increase our knowledge of the mechanics of homogenization of milk, various commercial and experimental valves were investigated to study the opportunity for cavitation during operation and to determine whether cavitation is necessary to achieve homogenization.

Literature regarding existing theories has been reviewed by Sommer *(7)* and Trout *(8)*. For purpose of reference other theories are defined as follows:

Shear—the break-up of fat globules due to high velocity within the homogenizing valve, whether this be accounted for by viscous shear at the boundary or by turbulent shear in the core.

Attenuation—the flattening and elongation of fat globules by acceleration at the valve entrance, followed by dispersion resulting from eddy motion.

Impact—the impingement of a stream of milk against a surface with sufficient velocity energy to break up the fat globules.

Loo *et al. (3)* reported a hydraulical analysis of the flow through a Cherry-Burrell 125 viscolizer valve. This indicated that cavitation should occur during operation. Valve clearances were determined for this analysis. A valve was modified in a manner to reduce opportunity for shear and impact but still produce cavitation. The modified valve gave more effective homogenization than the original valve when operated at the same pressures. They suggested that cavitation is one of the important factors in the homogenization of dairy products.

In a subsequent investigation, Loo and Carleton *(2)* used a single-piston 25 g.p.h. homogenizer to study the influence of back-pressure on the effectiveness of homogenization by the first-stage valve and concluded that back pressures higher than 500 p.s.i. reduced the effectiveness. When tiny sampling holes were drilled in homogenizer valves at various locations, a great dip in pressure (almost to atmospheric) occurred near the inner edge of the valve. Homogenization was virtually complete immediately beyond this location. Since cavitation will occur at any point where the pressure tends to drop below the vapor pressure, they considered the dip in pressure to indicate occurrence of cavitation. The homogenizing effectiveness of a knife-edged valve used with a Vickers 9-piston pump at 25 g.p.h. was compared with that of a conventional valve operated

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at the same flow rate with a Manton-Gaulin single-piston homogenizer. Under these conditions the knife-edged valve accomplished satisfactory homogenization of milk and concentrated milk at much lower pressures than did the conventional valve.

The impact theory was supported in results published by Doan and Minster *(1)*. In the first of two experiments described, variables other than those determining impact may have influenced the effectiveness of homogenization. Differences reported in the second experiment were related more to clumping than to reduction in size of the fat globules.

The report by Loo and Carleton that homogenization is virtually completed at 0.016 in. from the inner periphery of the valve is experimental evidence against impact.

EQUIPMENT AND METHODS

A 400 g.p.h. model CGD Manton-Gaulin 3-cylinder homogenizer, which delivered 410 ± 10 g.p.h. over a pressure range of 800 to 2,800 p.s.i., was used for the experiments reported (except one).

Tests were made with the following valves:

Manton-Gaulin—conventional Manton-Gaulin first-stage poppet-type valve.

Rounded-entrance—Manton-Gaulin valve with the beveled edge at the crevice entrance rounded to a radius of curvature of 0.1 in.

Ball—Manton-Gaulin valve seat with a steel ball $\frac{5}{8}$ in. in diameter as the valve.

Superhomo—Manton-Gaulin valve with Cherry-Burrell Superhomo replaceable caps, size 2, placed over the seat and valve. This arrangement was not entirely comparable with the Superhomo machine because of the slight difference in the valve-surface angle.

Multi-flo—Creamery Package single service homogenizing valve, size 400 gal., used with its 400 g.p.h. Creamery Package homogenizer instead of the Manton-Gaulin machine.

All experiments reported were conducted with mixed herd milk containing approximately 4% fat homogenized at about 140° F. Effectiveness of homogenization is expressed in terms of the U. S. Public Health Service (U.S.P.H.S.) index.

Valve clearances were measured by a dial gauge (reading to 0.0001 in.) in contact with the valve-rod cap-nut. As an example of the reproducibility obtained by this method, the standard deviation of the individual readings made in triplicate at each pressure for the water curve in Figure 1 was 0.0001 in.

A sampling device was constructed that enabled removal of test samples between the first and second stage valves. The device consisted of a rectangular loop of "extra strong" pipe, $\frac{3}{8}$ in. nominal diameter for the ends and bottom and $\frac{3}{4}$ in. for the top. To supply milk to the loop, the ends were connected to an adapter mounted between the first and second stage valve bodies. In taking a sample, the homogenizer was stopped and milk was removed through a valve that drained only the $\frac{3}{4}$ in. pipe. Before removal of the sample, sufficient milk

was passed through the system to assure that all of the sample was homogenized under the conditions recorded. A pressure gauge in the loop indicated the back pressure on the first stage valve. The homogenizer pressure gauge indicated the total pressure. The homogenizing pressure, therefore, was the pressure differential across the first stage valve.

Experiments were conducted with Venturi tubes used as a substitute for the homogenizer valve. Details regarding construction of the tubes are included in Figure 4. In some tests the discharge from the Venturi was fed back into the homogenizer inlet to permit circulation of the milk. A hydraulic piston was connected to the system so that static pressure could be applied to the fluid. For the circulation tests, dead-end spaces in the pressure gauge block and intake manifold were reduced by inserts. To minimize air occlusion, the homogenizer was filled step-wise as it was assembled for each test.

RESULTS AND DISCUSSION

Analysis of flow characteristics through a homogenizer valve. The flow characteristics through a Manton-Gaulin valve were examined theoretically to determine whether cavitation could occur. Bernoulli's equation was used to estimate the minimum pressure in the valve crevice. To arrive at values for this equation: *(a)* the velocity distribution across the valve face was established; *(b)* the Reynolds number across the valve face was calculated; then *(c)* an appropriate friction factor was estimated and used to calculate the head loss and, therefore, the pressure at any desired point. The results of this theoretical analysis showed that cavitation may exist in the valve.

(a) The velocity distribution across the valve face [Figure 2(A)] was calculated from the experimental relationship between valve clearance and pressure differential for the Manton-Gaulin valve (Figure 1). The equation of continuity:[5]

$$v = \frac{Q}{2\pi rc} \qquad (1)$$

shows that this velocity distribution has the form of a rectangular hyperbola. However, the presence of a vena contracta would cause a higher velocity immediately downstream from the entrance of the valve crevice than that indicated by Figure 2(A).

(b) The equation for the Reynolds number along the Manton-Gaulin valve face [Figure 2(B)] was developed as follows:

$$R_e = \frac{4vr_hd}{\eta} \qquad (2)$$

The hydraulic radius for the poppet valve (assumed 90°) is:

$$r_h = \frac{2\pi rc}{4\pi r} = \frac{c}{2} \qquad (3)$$

Substituting Equation 1 and Equation 3 into Equation 2 yields:

$$R_e = \frac{4d}{\eta} \cdot \frac{Q}{2\pi rc} \cdot \frac{c}{2} = \frac{Qd}{\pi r\eta} \qquad (4)$$

[5] Symbols used are given in Appendix A.

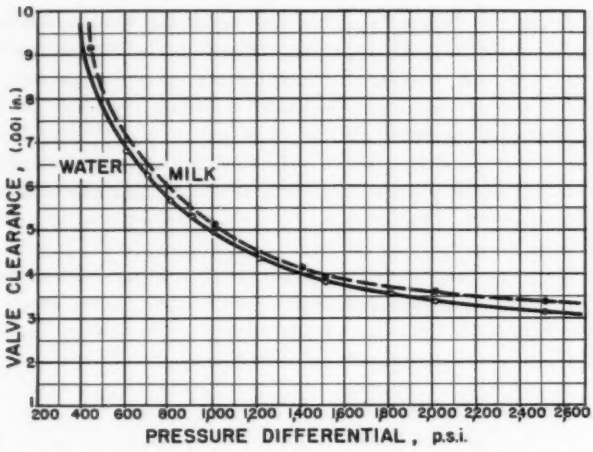


FIG. 1. Relation between pressure differential (homogenizing pressure) and valve clearance for the Manton-Gaulin first stage valve operated at 410 g.p.h. with water and milk.

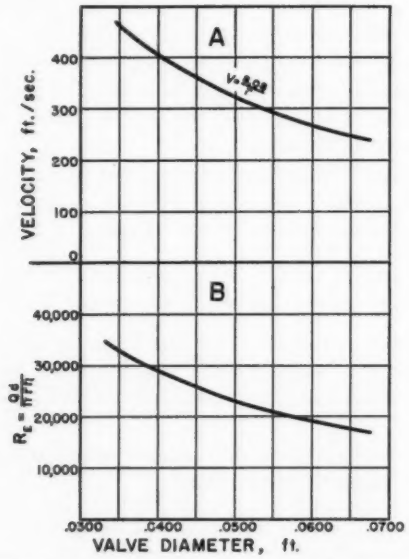


FIG. 2. Velocity distribution (A) and Reynolds number distribution (B) across the face of the Manton-Gaulin valve for a flow rate of 410 g.p.h. and valve clearance 0.0036 in.

Thus, Reynolds number is independent of the valve clearance. As long as the flow rate is constant, Reynolds number is not influenced by homogenizing pressure.

(c) For estimating the friction factor it was assumed that the flow was turbulent. This assumption appeared justified in view of the high Reynolds number,

the positive gradient from B to A (established below), a small variation in friction factor over a wide range of Reynolds number, and the sharp entrance to the valve.

In the absence of friction factors for radial flow in flat annuli, the factor was taken from the highest roughness for flow in pipes at the highest value of Reynolds number in the valve, i.e. 35,000 *(9)*. This was 0.018.

Fanning's equation, when made applicable to the homogenizing valve, has the following form:

$$\frac{dh}{dr} = -\frac{f\,Q^2}{4\pi^2 g\,c^3 r^5} \qquad (5)$$

The total head loss across the valve may be obtained by integrating between the limits A and B (Figure 3).

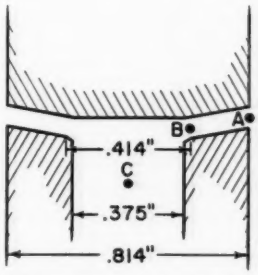


FIG. 3. Simplified diagram of the Manton-Gaulin valve.

$$h_{BA} = \frac{f\,Q^2}{4\pi^2 g\,c^3}\left(\frac{1}{r_B} - \frac{1}{r_A}\right) \qquad (6)$$

For a flow rate of 410 g.p.h. and a valve clearance of 0.0036 in. (homogenizing pressure 2,000 p.s.i.) this is 3,500 ft-lb/lb.

Bernoulli's equation was applied to find the pressure at B:

$$\frac{p_B}{d} + \frac{v^2_B}{2g} = \frac{p_A}{d} + \frac{v^2_A}{2g} + h_{BA} \qquad (7)$$

Since the opening at A is abrupt, the recovery of velocity energy is negligible. Therefore, the pressure at A can be taken as the pressure at the second stage valve. Because of the valve entrance condition, there must be a contraction coefficient, C_D, which shall be assumed to be 0.775 (see Appendix B). Substitution of appropriate valves into Equation 7 yields:

$$\frac{p_B}{63.3} + \frac{(608)^2}{64.4} = 0 + \frac{(238)^2}{64.4} + 3{,}500$$

$$p_B = -592 \text{ p.s.i.}$$

The negative pressure at B concurs with data of Loo *et al. (3)* that cavitation may exist in the valve.

Homogenization as influenced by valve design. Five commercial and experimental valves were tested for homogenizing effectiveness (U.S.P.H.S. index) at various pressures. The results are summarized in Table 1.

TABLE 1

Influence of valve design on effectiveness of homogenization[a]

No.	Valve	Homogenizing pressure *(p.s.i.)*									
		500		1,000		1,500		2,000		2,500	
		Valve clearance	U.S.P.H.S. index	Valve clearance	U.S.P.H.S. index	Valve clearance	U.S.P.H.S. index	Valve clearance	U.S.P.H.S. index	Valve clearance	U.S.P.H.S. index
		(in.)		*(in.)*		*(in.)*		*(in.)*		*(in.)*	
1	Manton-Gaulin	0.0093	>60	0.0052	25.0	0.0039	11.5	0.0036	6.7	0.00335	4.1
2	Manton-Gaulin with impact ring omitted	—	>60	0.0051	20.0	0.0038	9.8	0.0034	6.0	0.0032	2.8
3	Superhomo	—	>60	—	19.5	—	7.6	—	5.1	—	2.9
4	Multi-flo[b]	—	—	—	18.7	—	5.2	—	2.9	—	—
5	Ball	—	>60	0.0047	37.9	0.0038	24.5	0.0032	14.4	0.0030	10.8
6	Rounded-entrance	0.0060	>60	0.0038	20.0	0.0032	11.4	0.0031	5.4	0.0027	4.8

[a] Results are averages of 4 tests.

[b] Run on 400 g.p.h. Creamery Package homogenizer. Others were made with Manton-Gaulin pump delivering 410 g.p.h.

Comparison of Tests 1 and 2 indicates that impact is not an important mechanism for homogenization of milk by the Manton-Gaulin valve. Elimination of the impact ring did not reduce the effectiveness of homogenization even though the liquid stream emerging from the valve had about 10 times farther to travel before striking a surface. Since the interspace is filled with liquid, the dissipation of velocity energy due to the interactions of the fluids should have caused a decrease in homogenizing effectiveness if impact were important *(7)*.

Differences in results with the Manton-Gaulin, Superhomo, and Multi-flo valves cannot readily be explained in terms of cavitation. As pointed out by Sommer *(7)*, the superior effectiveness of the Multi-flo and Superhomo valves is more logically attributable to the greater opportunity for shear.

The ball valve may be considered similar to the knife-edged valve used by Loo and Carleton *(2)*. This valve gave homogenizing effectiveness comparable to the Manton-Gaulin valve when used in a 75 g.p.h. machine, but not when both were operated at 410 g.p.h. Calculations based on Equation 7 showed that cavitation could occur in both valves. The ball valve, however, reduced the opportunity for shear.

With the rounded-entrance valve, essentially no difference was observed in the adequacy of homogenization. The valve clearance was reduced, presumably because of reduction of vena contracta and increase of C_D (Appendix B).

Homogenization as influenced by back pressure. Table 2 shows the influence of back pressure on homogenizing effectiveness. From the calculations based on Equation 7, it is evident that if the second stage pressure (back pressure)

TABLE 2

Influence of back pressure on effectiveness of homogenization by the Manton-Gaulin and rounded-entrance valves

			Manton-Gaulin valve		Rounded-entrance valve	
Homogenizing pressure	Back pressure	Total pressure	Valve clearance	U.S.P.H.S. index	Valve clearance	U.S.P.H.S. index
(p.s.i.)	*(p.s.i.)*	*(p.s.i.)*	*(in.)*		*(in.)*	
500	0	500	0.0093	>60	0.0060	>60
500	200	700	0.00615	46.6	0.00445	38.5
500	500	1,000	0.00595	31.2	0.0044	29.8
500	1,000	1,500	——	——	0.0044	29.8
500	1,500	2,000	0.0063	25.0	0.0042	29.8
1,000	0	1,000	0.0052	25.0	0.0038	20.0
1,000	200	1,200	0.00445	18.9	0.00355	15.3
1,000	500	1,500	0.0043	13.5	0.00355	12.5
1,000	1,000	2,000	0.0044	13.5	0.00345	14.7
1,000	1,500	2,500	0.0044	11.2	0.0035	10.1
1,500	0	1,500	0.0039	11.5	0.0032	11.4
1,500	200	1,700	0.0038	7.1	0.00295	9.6
1,500	500	2,000	0.0038	8.0	0.00295	6.6
1,500	1,500	3,000	0.0038	5.6	0.0029	7.5
2,000	0	2,000	0.0036	6.7	0.0031	5.4
2,500	0	2,500	0.00335	4.1	0.0027	4.8

exceeds 600 p.s.i., cavitation should be suppressed. In Table 2, therefore, cavitation should be a factor with zero back pressure, but not at pressures of 600 p.s.i. and above.

If cavitation is important, adequacy of homogenization should decrease under this latter condition. Since a decrease did not occur, the results do not support the cavitation theory.

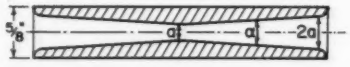
The coefficient of correlation between valve clearance and the U.S.P.H.S. index was 0.81 for the Manton-Gaulin valve and 0.90 for the rounded-entrance valve. These values are highly significant ($p < 0.1\%$). Since the flow rate is constant, the same correlation must also exist between velocity and the U.S.P.H.S. index.

The results indicate that the mechanism of homogenization depends upon velocity or velocity gradient. The impact, shear, or attenuation theories satisfy this condition, but in view of experimental results reported in Table 1 and by Loo and Carleton *(2)*, the impact theory appears untenable.

Homogenizing characteristics of Venturi tubes. Since the cavitating Venturi tube lends itself to mathematical analysis, experiments were conducted with several tubes designed for prevention of cavitation at different back pressures (Figure 4). The lucite Venturi was constructed to permit visual observation of cavitation. Figure 5 illustrates the suppression of cavitation by applying back pressure.

The results of tests to study the homogenizing effect of the Venturi tubes are summarized in Table 3. With one pass, no measurable homogenization was observed, either with or without cavitation. In recirculation tests involving 13 passes, more effective homogenization was noted under cavitating conditions. The difference is statistically significant ($p < 0.1\%$). The results of 80 passes (where the surface area per unit volume of fluid approaches that of a commercial valve) showed no statistically significant difference between cavitation and non-cavitation.

To interpret these results, consideration should be given to the nature of flow in a Venturi tube. When cavitation occurs, the main body of liquid, after passing through the throat of the Venturi, flows as a free but usually very turbulent jet, surrounded by a foaming mixture of liquid and vapor bubbles.



NO.	MATERIAL	THROAT DIAMETER a_t (inches)	INCLUDED ANGLE α	MAXIMUM VELOCITY (f.p.s.)	CRITICAL PRESSURE (p.s.i.)
1	Brass	.093	7°	333	770
2	Brass	.143	7°	140	125
3	Brass	.375	7°	20	—
4	Lucite	.093	7°	333	770

FIG. 4. Design data of the Venturi tubes. The critical pressure is the back pressure at which cavitation theoretically should be prevented.

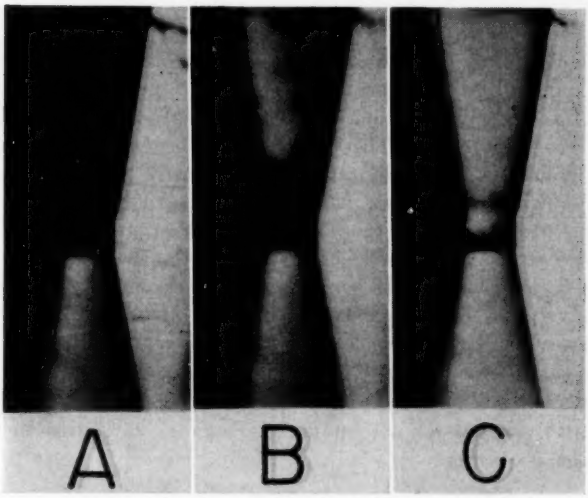


FIG. 5. Suppression of cavitation by application of back pressures of A—0 p.s.i.; B—600 p.s.i.; C—1,000 p.s.i. Flow was from bottom to top.

Therefore, the recirculation tests provided greater opportunity for the turbulent core to be subjected to the region of cavitation. However, greater chances were also provided for subjection to both viscous and turbulent shear.

Although more effective homogenization was obtained in the 13-pass tests when cavitation occurred, it should not be concluded that this resulted from cavitation per se. Increased turbulent shear could accompany cavitation because of the high local velocity gradients accompanying bubble collapse and also because of the increase in velocity necessitated by the reduction in area for flow of the liquid in the presence of the vapor.

Results obtained with Venturi tubes No. 1 and 2 under cavitating conditions might be influenced by intensity of cavitation. However, the results of the back-pressure experiments in Table 2 do not support such an interpretation of the data for the Manton-Gaulin valve.

TABLE 3

Homogenizing effects of Venturi tubes

Venturi tube no.	Back pressure	Cavitation	U.S.P.H.S. index after:		
			1 pass	approx. 13 passes	approx. 80 passes
	(p.s.i.)				
1	0	yes	>60	11.1	10.6
	1,100	no	>60	27.4	12.2
2	0	yes	>60	56.9	—
	1,100	no	>60	>60	—
3	0	no	>60	>60	—
	1,100	no	>60	—	—

SUMMARY

The valve clearances in a Manton-Gaulin homogenizing valve operating with both water and milk were determined for various pressures and used in the calculation of velocity distribution, Reynolds number, and minimum theoretical pressure in the valve crevice. The analysis showed that cavitation could occur.

Results obtained in homogenization of milk with several commercial and experimental valves are discussed with reference to theories of homogenization. Effective homogenization was accomplished in the Manton-Gaulin and a rounded-entrance valve even when sufficient back pressure was applied to prevent cavitation. The correlation between valve clearance and the U.S.P.H.S. index indicates that the mechanism of homogenization involves velocity or velocity gradient. Tests with Venturi tubes provided conditions where cavitation occurred, yet homogenization was relatively ineffective in comparison with that accomplished by commercial valves.

The results reported do not support either the cavitation or the impact theories of homogenization.

APPENDIX A

Symbols

A — area (ft.^{2})
c — valve clearance (ft.)
C_D — contraction coefficient
d — density (lb/ft^3)
f — friction factor
g — gravitational constant (32.2 ft/sec^2)
h — head loss (ft-lb/lb)
p — pressure (p.s.f.)
Q — flow rate (c.f.s.)
r — radius (ft.)
r_h — hydraulic radius — $\frac{\text{cross-sectional area of flow path (ft.}^2\text{)}}{\text{wetted perimeter (ft.)}}$
R_e — Reynolds number
v — velocity (ft/sec)
η — viscosity (lb/sec-ft.)

APPENDIX B

In flow through a sharp-edged orifice, there is a contraction of the issuing jet, due to the change in direction of flow lines. The percentage reduction in area of the jet as compared with the orifice is known as the coefficient of contraction (C_D).

C_D must lie between 0.61 (sharp entrance) and 1.0 (rounded entrance) *(4)*. Since the homogenizer valve crevice is sharp on one side only, an intermediate value may be expected.

The C_D for the Manton-Gaulin valve was estimated as follows: Assume v_c negligible compared to v_B, and $p_B = 0$.

$$\frac{p_c}{d} = \frac{v_B^2}{2g} = \frac{Q^2}{2gC_D A^2}$$

Hence:

$$C_D = \frac{Q}{A}\left(\frac{d}{2gp_c}\right)^{1/2} = 0.775$$

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A STUDY OF THE VOLATILE FRACTION ISOLATED FROM OXIDIZED MILK FAT. I. ISOLATION, PRELIMINARY CHARACTERIZATION, AND CHROMATOGRAPHIC SEPARATION OF THE VOLATILE FRACTION[1]

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Relatively little information is available in the literature concerning the nature of the compounds responsible for the oxidized flavor of milk fat. The earlier literature indicates that saturated aldehydes are mostly responsible for this flavor *(9)*. In more recent years evidence was obtained of the presence of several unsaturated carbonyl compounds in oxidized fats, oils, fatty acids, and in their esters *(1, 2, 3, 4, 7, 10, 13, 14)*. These compounds exhibit characteristic ultraviolet absorption maxima *(5)*. Van der Waarden *(15)* postulated that methyl ketones and unsaturated aldehydes with no conjugation contributed to the off-flavors of cold storage butter. Keeney and Doan *(8)* demonstrated the contribution of ketones to the flavor of oxidized milk fat.

The present study was undertaken to obtain more information concerning the nature of the compounds responsible for the oxidized flavor in milk fat. These compounds were removed from milk fat by a deodorization procedure similar to those used in the vegetable oil industry. The characterization of these volatile substances was essentially based on determination of carbonyl groups and on the ultraviolet absorption spectra. On the basis of ultraviolet absorption maxima, these compounds were further separated by chromatography in an attempt to localize those substances that contribute to the oxidized flavor of milk fat.

MATERIALS AND METHODS

Preparation of oxidized fat. Milk fat was prepared from fresh butter melted at 55-60° C. The fat was decanted from the aqueous layer and filtered. It was then dried and degassed by heating at 100° C. under a pressure of 12 mm. of mercury for 10 minutes. It was stored at –18° C. in completely filled glass bottles for not more than 2 months. The milk fat was oxidized under the following conditions:

1. Holding at 100° C. for 24 hours in layers 2.5 cm. thick in covered stainless steel trays 5 cm. high.
2. Holding at 40° C. for 1 month in layers 0.5 cm. thick in closed Petri dishes 1.5 cm. high, in the dark.
3. Holding at 25° C. for 1 month in layers 0.5 cm. thick in closed Petri dishes 1.5 cm. high, in the dark.
4. The same as No. 3 except held in daylight.

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Deodorization of the fat. The fat was deodorized immediately after oxidation with a steam distillation apparatus as described by Riemenschneider *et al. (12)*. A quantity of 200 ml. of fat was used in each run. The fat was deodorized at 100° C. under a pressure of 4 mm. of mercury for 1 hour. The distillate was collected in a dry ice-acetone trap. It was later melted and extracted with 5 ml. of Skellysolve B purified according to Graff *et al. (6)* for spectral analysis.

Analysis. The carbonyl content was determined in duplicate by Lea's aldehyde method *(9)* in the fat before and after deodorization (2.5-g. samples), in the Skellysolve extract (1-ml. samples), and in the remaining water distillate after extraction (5-ml. samples). Blank values were 0.19μM per sample. Peroxide values were determined by Lea's peroxide method *(9)*. The Kreis test according to Pool and Prater *(11)* was used as modified in this laboratory *(16)* by measuring the transmission at the adsorption maxima 545 and 450 mμ. The ultraviolet absorption of Skellysolve extracts was determined with a Beckman DU spectrophotometer in properly diluted samples using 1-cm. cells. The oxidized flavor of milk fat was evaluated organoleptically by taste and smell. Aqueous distillates derived from deodorization and Skellysolve extracts were examined by smell only.

Chromatographic separation. For chromatographic separation, larger quantities of volatile fraction were obtained by deodorizing 4 l. of oxidized fat in six runs, using 25, 20, 15, and 10 ml. of Skellysolve B successively to extract the combined distillates. The total extract was used.

Adsorbents. The following adsorbents were tried: Celite analytical Filteraid (Johns-Manville), magnesium sulfate dried cp. $MgSO_4 \cdot 3H_2O$ (Merck), Alumina Adsorption 80-200 mesh (Fisher), Silicagel 28-200 mesh (Davison), and Floridin XXS. Columns were of two sizes: 90 × 22 mm. and 280 × 43 mm. For the small size, 10 ml. of extract diluted 1:20 with Skellysolve was eluted with 250-300 ml. of Skellysolve under a pressure of 50 mm. of mercury. The approximate time per run was 5 hours. For the large size, these figures were 100 ml. of extract, 2,000-3,500 ml. of eluate, 200 mm. of mercury, and 8 hours. Once the technique was established with small columns, larger ones were used to obtain sizeable fractions.

The columns were filled with a thin slurry of the adsorbent and Skellysolve B until the desired height was obtained. The solution to be separated was added to the column when the solvent level was approximately 1 mm. above the adsorbent level. At this point the eluent was allowed to percolate through the column under pressure. Fractions were collected and examined for their absorption in the ultraviolet region and for oxidized flavor.

RESULTS

Organoleptic evaluations revealed that deodorization removed the oxidized flavor from the fat. Although the aqueous distillates smelled strongly oxidized, no oxidized flavor could be detected in the deodorized fat. Skellysolve extraction removed the oxidized flavor from the aqueous distillate.

Upon melting the distillate, a small quantity of greenish-yellow oil, soluble in

TABLE 1

Removal of "aldehydes" from oxidized fat by deodorization

Conditions of oxidation	100° C. in the dark	40° C. in the dark	25° C. in the dark	25° C. in the daylight
Fat before deodorization *(ml.)*	200	200	200	200
Aldehyde per gram *(μM)*	5.86	0.39	0.13	0.18
Fat after deodorization *(ml.)*	200	200	200	200
Aldehyde per gram *(μM)*	1.17	0.27	0.11	0.14
Skellysolve extract *(ml.)*	5.0	5.2	5.2	5.2
Aldehyde per ml. *(μM)*	102.63	0.43	0.12	0.29
Aldehyde recovered *(%)*	60.1	10	17	21
Distillate after extraction *(ml.)*	53	64	67	71
Aldehyde per ml. *(μM)*	5.71	0.15	0.05	0.08
Aldehyde recovered *(%)*	35.4	44	92	78

Skellysolve or alcohol, separated from the water. This oil contained most of the oxidized flavor and carbonyl compounds.

Table 1 shows data on the removal of carbonyl compounds by deodorization. Under milder conditions of oxidation, the portions of the carbonyl compounds less volatile and less soluble in Skellysolve were relatively larger. Percentage recovery of "aldehydes" removed from fat oxidized at 100° C. was 95; 60% was found in the Skellysolve extract and 35% in the distillate after extraction. Percentages recovery in the milder oxidation treatments are not accurate because of the small aldehyde values and relatively large error involved. Peroxide and Kreis values were run on the samples obtained by deodorization of fat oxidized below 100° C. Deodorization caused a 10-30% decrease in the peroxide value of the fat. No decrease was found in the Kreis value. The Skellysolve extracts were negative for both of these values.

The volatile Skellysolve soluble fraction was studied further by means of ultraviolet absorption. The data obtained are illustrated in Figure 1. Fresh fat showed a maximum between 225 and 230 mμ. Oxidation of the fat caused an increase in absorption; the original maximum shifted to 215-220 mμ and a second maximum developed at 260-265 mμ. These maxima indicate the presence of conjugated monenecarbonyl compounds and conjugated dienecarbonyl compounds, respectively *(5)*.

Volatile fractions from fats oxidized at 100° C., diluted to various degrees in Skellysolve, were stored at 40° C. for 6 weeks in flasks closed with aluminum caps in the dark. Ultraviolet absorption data expressed as extinction (Ext.) before and after storage are presented in Table 2. Before storage the ratio Ext. 215 mμ : Ext. 265 mμ of the fresh Skellysolve solutions was not affected by change in concentration. After storage, the ratio showed marked increase in the case of concentrated solutions, but with dilute solutions it stayed practically the same. The increase in ratio was caused by a decrease in extinction at 265 mμ. The Ext. at 215 mμ remained fairly constant. The slight increase in Ext. generally found after storage was probably caused by some evaporation of Skellysolve. Dilution (1:20) of Skellysolve extracts from deodorization distillates assured proper stability of the volatile fraction for refrigerator storage.

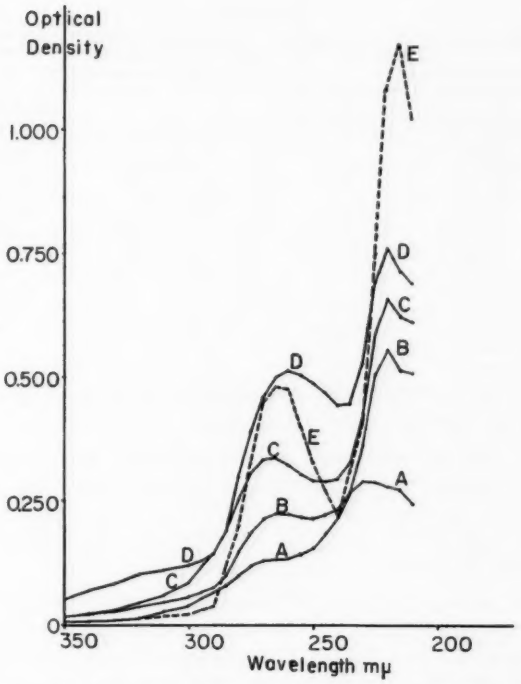

FIG. 1. Ultraviolet absorption of the volatile fraction in Skellysolve solution.

	Method of oxidation	Dilution of the volatile fraction	Aldehyde (*μM per ml. dilution*)
A =	fresh fat	1:10	0.0
B =	25° C. (dark)	1:10	0.012
C =	25° C. (daylight)	1:10	0.029
D =	40° C. (dark)	1:10	0.043
E =	100° C. (dark)	1:1,000	0.103

An attempt was made to separate by chromatography the two ultraviolet absorption maxima at about 215 and 265 mμ exhibited by the volatile fraction from milk fat oxidized at 100° C.

Among the different adsorbents tried, Celite and $MgSO_4$ (Merck) were found to effect separation of the two maxima at about 215 and 265 mμ. When Skellysolve was used as the solvent with these adsorbents, the maximum at 215 mμ was eluted at a greater velocity than the maximum at 265 mμ. Hence, the first fractions collected consisted of one maximum at 215 mμ followed by a mixture, and the last fractions showed one maximum at 265 mμ. The columns were of limited capacity. The quantity of volatile fraction run through one column could be increased up to a certain point, beyond which the separation became less efficient. Only one run could be made on the same column. Celite proved to be slightly

TABLE 2

Effect of storage on ultraviolet absorption of volatile fraction

Skelly solutions fresh			After 6 weeks at 5° C.			Increase in
Ext.[a] 265 mμ	Ext. 215 mμ	Ext. 215: Ext. 265	Ext. 265 mμ	Ext. 215 mμ	Ext. 215: Ext. 265	Ext. 215: Ext. 265
491	1238	2.52	182	1548	8.51	5.99
427	1139	2.67	117	1186	10.13	7.46
366	929	2.54	170	871	5.12	2.58
23.6	60.1	2.55	23.3	61.0	2.62	0.07
7.8	19.0	2.44	8.25	21.2	2.57	0.13
4.27	11.39	2.67	4.68	12.98	2.77	0.10
1.75	4.46	2.55	1.96	4.98	2.54	−0.01
0.476	1.161	2.44	0.533	1.321	2.48	0.04
0.175	0.446	2.55	0.198	0.449	2.52	−0.03

[a] Ext. = Extinction.

more efficient than $MgSO_4$. Since the latter was not available from various sources in uniform quality, Celite was adopted.

When Skellysolve was used with alumina, silicagel, and Floridin, no elution of the volatile fraction could be achieved. Alcohol was capable of eluting the volatile fraction from these adsorbents. However, no separation of the two maxima at 215 and 265 mμ could be effected with this solvent. Mixtures of alcohol and Skellysolve produced little or no separation.

Table 3 gives data on the chromatographic separation of the volatile fraction in Skellysolve solution with Celite. Recoveries were calculated on the basis of optical densities at 215 and 265 mμ. The eluate was examined continuously for ultraviolet absorption and oxidized odor. The fraction collector was changed when considerable shift in the ratio O.D. 215 : O.D. 265 mμ was observed. The final fraction was eluted with purified alcohol to determine whether any volatile fraction was left in the column. Fractions (accumulated from several runs) were concentrated by distillation under atmospheric pressure with a column 20 cm. long, and the residues were rechromatographed. Approximately 20% was lost in the distillate, most of which could be recovered by redistilling the latter. The greatest part of material responsible for the oxidized odor was recovered in the first fractions of Runs I and II. Later fractions had a weaker odor and were not typically oxidized. An oxidized odor was also observed in the eluate just preceding the first fraction in which practically no ultraviolet absorption was present. The capacity per Celite column of 280 × 43 mm. corresponded to the volatile material obtained from approximately 0.3 l. of milk fat oxidized at 100° C.

SUMMARY AND CONCLUSIONS

The results of this study indicate that carbonyl compounds appear to be related to the oxidized flavor in milk fat. The volatile fraction concentrates in Skellysolve that possessed the oxidized flavor contained carbonyl compounds, but no peroxides or epoxy compounds were present. Only part of the carbonyl compounds from oxidized milk fat was recovered in these concentrates; the

TABLE 3

Ultraviolet absorption characteristics and recoveries of fractions obtained by chromatography on Celite

Runs	Fractions	Eluate	O.D.[e] 215 mμ / O.D. 265 mμ	O.D. 265 mμ recovered	O.D. 215 mμ recovered
		(ml.)		*(%)*	*(%)*
I[a]	1	25	7.5	20.7	59.1
	2	25	2.0	26.4	20.2
	3	75	0.25	27.3	2.6
	4	100	0.02	8.6	0.1
	5	25	2.64	7.1	7.1
	Total	250		90.1	89.1
II[b]	1	200	79.3	0.9	26.6
	2	200	19.9	4.1	30.4
	3	300	3.6	16.0	21.7
	4	600	0.48	33.3	6.0
	5	1,000	0.0	27.7	0.0
	6	150	0.0	3.0	0.0
	Total	2,450		85.0	84.7
III[c]	1	125	42.2	6.8	13.7
	2	125	84.9	7.8	30.6
	3	250	39.8	18.0	34.4
	4	375	14.7	16.8	11.7
	5	1,000	2.0	19.6	1.9
	6	150	0.0	7.0	0.0
	Total	2,025		76.0	92.3
IV[d]	1	250	∞	0.0	22.1
	2	125	3.4	2.5	18.3
	3	375	0.71	18.6	28.5
	4	250	0.31	13.2	8.7
	5	1,750	0.0	41.4	0.0
	6	125	0.0	8.2	0.0
	Total	2,875		83.9	77.6

[a] Column 90 × 22 mm., 10 ml. Skellysolve solution: O.D. 265 mμ = 22.5, O.D. 215 mμ = 59.0, O.D. 215 : O.D. 265 mμ = 2.62.

[b] Column 280 × 43 mm., 100 ml. Skellysolve solution: O.D. 265 mμ = 21.6, O.D. 215 mμ = 57.9, O.D. 215 : O.D. 265 mμ = 2.68.

[c] Column 280 × 43 mm., 100 ml. concentrated fraction 2 : O.D. 265 mμ = 3.3, O.D. 215 mμ = 69.4, O.D. 215 : O.D. 265 mμ = 21.3.

[d] Column 280 × 43 mm., 100 ml. concentrated fraction 4 : O.D. 265 mμ = 28.0, O.D. 215 mμ = 13.1, O.D. 215 : O.D. 265 mμ = 0.47.

[e] O.D. = optical density.

other parts of the carbonyl compounds remaining in the fat and in the distillate involved in the procedure did not appear to contribute to the oxidized flavor. The relatively much smaller percentages of volatile and Skellysolve soluble carbonyl compounds obtained under the milder conditions compared with oxidation at 100° C. (Table 1) indicate a different composition of the total mixture of carbonyl compounds. The volatile fraction concentrates exhibited two characteristic absorption maxima at 215-220 mμ and at 260-265 mμ, indicating the presence of conjugated monenecarbonyl compounds and conjugated dienecarbonyl compounds, respectively *(5)*.

A close relationship between the development of oxidized flavor, carbonyl compounds, and characteristic absorption maxima was observed during autoxidation of milk fat. Changes in ultraviolet absorption were distinct in early stages

when oxidized flavor or carbonyl compounds were still negative or below the accuracy limits of the methods. Hence, ultraviolet absorption characteristics may prove to be sensitive tools in fat oxidation studies.

The maximum at 260-265 mμ in the volatile fraction concentrates from fat oxidized at 100° C. decreased during storage at 4° C. The ratio of optical densities at 215 mμ and 265 mμ increased from approximately 2.5 to 10. This indicates that the diene compounds are less stable than the monene compounds. The change was prevented by diluting to 1:20 or more with Skellysolve.

Compounds present in volatile fraction concentrates could be separated by chromatography on Celite on the basis of ultraviolet absorption characteristics. The oxidized flavor was recovered in the first fractions, together with compounds absorbing at 215 mμ (monenecarbonyl compounds) and in the eluate just preceding these compounds. This indicates that compounds with no appreciable absorption in the ultraviolet region also contribute to oxidized flavor. Further studies made to characterize the above fractions will be reported in the second part of this article.

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THE RELATIONSHIP OF EGG YOLK AND GLYCEROL CONTENT OF DILUTERS AND GLYCEROL EQUILIBRATION TIME TO SURVIVAL OF BULL SPERMATOZOA AFTER LOW TEMPERATURE FREEZING[1]

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Many variables related to diluter composition and processing of bull semen for low temperature freezing and storage have not been adequately studied as to their effects on sperm survival.

Polge and Rowson *(9)* used a diluter which in its final combination contained 25% egg yolk, 10% glycerol, and 2.94% sodium citrate dihydrate. Dunn and Hafs *(2)* reported that egg yolk diluters in a final combination containing 25% egg yolk and 10% glycerol were superior to other diluters containing 27.5% and 50% egg yolk. Kinney and VanDemark *(4)* found that diluters containing 16 and 24% egg yolk (sodium citrate constant) were superior to a diluter containing 32% egg yolk.

Workers at Illinois *(4, 5)* evaluated egg yolk diluters (25%) containing 4, 6, 8, and 10% of glycerol and reported that the diluters containing 6 and 8% glycerol were superior to the other diluters as judged by percentage of motile sperm after freezing and thawing. Graham and Marion *(3)*, also using a 25% egg yolk diluter, found 10% of glycerol in a diluter to be superior to either 15 or 20%. Cragle and Myers *(1)* studied diluters with glycerol levels varying from 2 to 14% (egg yolk level constant at 25%) and reported that 7.6% glycerol was the optimum level.

Polge and Rowson *(9)* recommended that semen in egg yolk diluters be equilibrated for 15 to 20 hours with glycerol before freezing but gave no data to support this recommendation. Miller and VanDemark *(6)* studied 2-, 6-, and 18-hour glycerol equilibration times with egg yolk diluters and found the 6-hour period to be slightly better than the other two periods as judged by percentage of motile sperm after freezing. Cragle and Myers *(1)*, also using egg yolk diluters, found that a glycerol equilibration time of 14.9 hours was best as compared with other times ranging from 4 to 28 hours. O'Dell and Almquist *(8)*, using a heated skimmilk diluter, did not find significant differences in percentage of motile sperm after freezing when using 0.5-, 4-, and 18-hour glycerol equilibration periods.

In these references and in the experimental work presented in this paper both egg yolk and glycerol percentages were expressed on a volume basis.

As yet studies have not been reported in which the interactions among diluter egg yolk content, glycerol content, and glycerol equilibration time, as they affect percentage of motile sperm after freezing, can be evaluated. Such studies are reported in this paper on the use of bull semen with egg yolk–citrate diluters.

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EXPERIMENTAL PROCEDURES AND RESULTS

Semen from Holstein and Guernsey bulls regularly used in artificial breeding was used in these experiments. The semen was diluted 1:10 with the initial diluter containing egg yolk and sodium citrate at 30° C., and then cooled to 5° C. over a 3- to 4-hour period. The glycerol part of the diluter was added in three portions at intervals of 15 minutes, adding 20% of the glycerol diluter initially, 30% at 15 minutes, and 50% at 30 minutes. The final dilution rate of the semen was 1:20. This procedure for addition of the glycerol part of the diluter allowed equal percentage increases in glycerol content of the diluted semen with each addition of glycerol diluter. One-ml. portions of diluted semen were put into 1.6-ml. molded Pyrex glass ampoules and sealed with an oxygen-gas fishtail burner. The glycerolated semen was frozen at the rate of 1° C. per minute from 5° to –15° C., and at the rate of 3° to 4° C. per minute from –15° to –75° C., and was stored at a temperature of at least –75° C. in trays of ethanol. Initial examination of experimental semen samples was made about 12 hours after the end of freezing procedures. The percentage of motile sperm was used as the criterion of spermatozoan viability *(7)*. In all instances the initial diluter and the glycerol portion of the diluter contained egg yolk and sodium citrate dihydrate.

Experiment 1. Interactions among egg yolk content of diluters, glycerol content of diluters, and glycerol equilibration time. A factorial experiment was designed to use three levels of egg yolk (18.4, 23.0, and 27.6%), three levels of glycerol (6, 8, and 10%), four glycerol equilibration times (2, 6, 12, and 18 hours), and six semen samples. Sodium citrate levels were kept relatively constant at 2.175% in both the initial and the glycerol diluter. However, both egg yolk and sodium citrate levels deviated slightly from the stated levels since, in preparing the glycerol portion of the diluter, glycerol was added in varying amounts to the initial diluter, thus changing the proportions of egg yolk and sodium citrate in this portion. Table 1 presents the actual levels of egg yolk achieved in all instances in the final diluter, but for purposes of analysis it was considered that there were only three levels of egg yolk. Semen samples were thawed in tap water (approximately 22° C.) for examination.

There were 215 degrees of freedom in the data. An analysis of variance indicated that there were statistically highly significant effects ($P < 0.01$) due to egg yolk levels, glycerol levels, glycerol equilibration times, and semen samples. Statistically significant effects ($P < 0.05$) are attributable to the interaction between egg yolk and glycerol levels and a triple interaction among egg yolk levels, glycerol levels, and semen samples. No effects attributable to the interaction of glycerol equilibration time with other variables approached statistical significance. Evidence of the interaction between egg yolk and glycerol levels may be seen in Table 1. Glycerol content of 6% was superior for the two lowest levels of egg yolk at all glycerol equilibration times and the 8% of glycerol was uniformly superior for the highest level of egg yolk. The data uniformly showed an increase in percentage of motile sperm as the glycerol equilibration time increased.

TABLE 1

The effect of egg yolk level, glycerol level, and glycerol equilibration time on percentage of motile sperm after freezing to −75° C. (average of six semen samples)

Egg yolk level	Glycerol level	Hours of equilibration time				Mean
		2	6	12	18	
(%)	*(%)*	*(Motile sperm, %)*				
18.8	6	29.2	35.8	43.0	44.2	38.0
18.4	8	24.2	37.2	38.7	39.7	34.9
18.0	10	21.8	29.0	32.2	37.5	30.1
23.5	6	32.3	40.2	43.2	43.3	39.8
23.0	8	29.5	30.8	37.8	39.7	34.5
22.5	10	22.3	22.7	34.5	36.7	29.0
28.2	6	22.3	23.5	29.2	29.5	26.1
27.6	8	26.5	28.3	33.7	34.2	30.7
27.0	10	19.5	21.3	23.3	30.7	23.7
	Mean	25.3	29.9	35.1	37.3	31.9

The interaction found between egg yolk and glycerol levels was more completely investigated in Experiment 2. Since there was no evidence of interactions involving glycerol equilibration time, this variable was omitted in Experiment 2.

Experiment 2. Interaction between egg yolk and glycerol content of diluters. A second factorial experiment was designed with four levels of egg yolk (15, 20, 25, and 30%), five levels of glycerol (5, 6, 7, 8, and 9%), three periods of storage at 5° C. following thawing (0, 1, and 3 days), and with ten semen samples. Sodium citrate dihydrate was kept at a constant level of 2.175% in both the initial and the glycerol diluter. The egg yolk content was also adjusted so that the initial and the glycerol-containing diluters contained the amounts of egg yolk indicated. Semen samples were equilibrated for 18 hours with glycerol before freezing, and after freezing they were thawed in water at 5° C. for examination. Motility ratings were made initially after thawing (0 day) and again after storage at 5° C. for 1 and 3 days.

Data are presented in Table 2. The total degrees of freedom in the data of this experiment were 599. An analysis of variance of these data made with only zero day data showed statistically highly significant effects ($P < 0.01$) due to egg yolk levels, glycerol levels, semen samples, and interaction between egg yolk and glycerol levels. Figure 1 shows more clearly the nature of the interaction between egg yolk and glycerol levels with data for zero day. As the level of egg yolk increased, correspondingly higher levels of glycerol were required for optimal sperm survival. For the 30% egg yolk level, it seems that a level of glycerol higher than 9% would be required for best results. The diluter giving the highest percentage of motile sperm on either days 0, 1, or 3 of storage at 5° C. contained 20% egg yolk and 7% glycerol.

An estimate of random variation associated with the estimation of percentage of motile sperm may be obtained from the analysis of variance of the two experiments. The total degrees of freedom and the sums of squares of all nonsignificant sources of variation in both experiments were pooled and the mean square

TABLE 2

The effect of egg yolk and glycerol levels in diluters on percentage of motile sperm after freezing to –75° C. and on storage at 5° C. (average of ten semen samples)

Egg yolk	Glycerol	Day of semen examination		
		0	1	3
(%)	*(%)*	*(Motile sperm, %)*		
15	5	39.5	20.3	3.0
	6	42.3	24.4	5.4
	7	42.2	23.5	5.3
	8	41.2	20.7	5.8
	9	39.6	20.8	5.8
	Mean	40.9	21.9	5.1
20	5	38.7	21.1	4.8
	6	43.0	26.5	6.5
	7	44.4	29.5	7.8
	8	41.5	24.7	7.4
	9	39.2	22.3	5.5
	Mean	41.4	24.8	6.4
25	5	31.6	16.8	1.0
	6	37.6	23.6	4.1
	7	42.4	24.8	7.0
	8	42.8	27.1	6.9
	9	39.4	23.6	3.3
	Mean	38.8	23.2	4.5
30	5	17.9	8.3	0.7
	6	25.9	15.2	1.6
	7	33.7	20.3	4.1
	8	38.5	20.7	4.8
	9	41.6	23.3	5.6
	Mean	31.5	17.6	3.4

was calculated as 47. Thus 6.9 is the standard error of a single estimate of percentage of motile spermatozoa. This figure suggests that the percentage of motile spermatozoa can be estimated to the nearest 5%, and it would be an interesting figure to compare among experimental workers making estimates of percentage of motile spermatozoa.

DISCUSSION

A marked interaction between egg yolk and glycerol levels as affecting sperm survival after freezing was shown by these data. This had not been reported previously. This observation suggests that the egg yolk in some manner "tied up" part of the glycerol, making the requirement for glycerol higher as the egg yolk content was increased. In evaluating this interaction between egg yolk and glycerol levels no advantage was found in the use of either 1- or 3-day storage data (5° C.) over the use of zero-day data (immediately upon thawing the samples).

Data relative to the effect of glycerol equilibration time on sperm revival after freezing are not in agreement among the various workers *(1, 6, 8, 9)*. The reason for this disagreement is not apparent. Our procedure in relation to diluter composition and glycerol equilibration time differs in one respect, at least, from the methods of the other workers. Egg yolk, in our experiments,

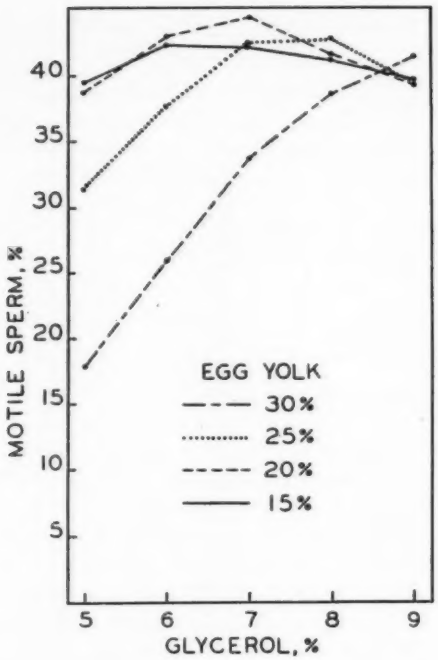

FIG. 1. Interaction between egg yolk and glycerol levels in semen diluters on the percentage of motile spermatozoa after freezing to −75° C.

was common both to the initial diluter and to the glycerol-containing portion of the diluter. In the references cited, egg yolk was a constituent of the initial diluter only, the glycerol being added in a citrate buffer. The above suggested "tying up" effect of egg yolk on glycerol might also be a factor in equilibration time since, in this instance, the egg yolk is a part of the glycerol-containing diluter.

Our findings relative to optimal egg yolk and glycerol levels for diluters to be used with frozen semen agreed well with those of other workers *(1, 2, 3, 4, 5, 9)*.

SUMMARY

In a factorial design experiment with three levels of egg yolk (18.4, 23.0, and 27.6%), three levels of glycerol (6, 8, and 10%), and four glycerol equilibration times (2, 6, 12, and 18 hours) there was a progressive increase in sperm survival in all diluter treatments after freezing to −75° C. as the glycerol equilibration time was increased from 2 to 18 hours. A significant interaction between egg yolk and glycerol levels also was demonstrated. There were no significant interactions involving glycerol equilibration time and the other variables.

In a second factorial design experiment, four egg yolk levels (15, 20, 25, and 30%), five glycerol levels (5, 6, 7, 8, and 9%), and three storage times at 5° C. (0, 1, and 3 days) after thawing were used. A highly significant interaction of egg yolk and glycerol levels was again demonstrated. As the egg yolk level was increased, the glycerol level had to be increased also for optimal sperm survival after freezing. A diluter containing 20% egg yolk and 7% glycerol gave best sperm survival after freezing.

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UREA AS A PROTEIN EXTENDER FOR LACTATING COWS[1]

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Various protein supplements have been used to balance the roughage ration of farm mammals to secure economical gains and efficient milk production. In recent years the use of urea as a protein extender has been given considerable emphasis. The literature concerning the utilization of urea by ruminants has been reviewed by Reid *(7)*; however, subsequent to the preparation of the review, Thompson *et al. (11)* reported that urea and cottonseed meal were of comparable value for milk production under the conditions of their experiment.

The object of the experiment reported here was to compare the milk-producing value of a low protein roughage ration supplemented with *(a)* corn and urea and *(b)* soybean oil meal, and also to observe the effectiveness of the nitrogen supplement when fed by two different methods.

PROCEDURE

Ten cows (nine Holstein and one Guernsey) were fed a protein depletion ration consisting of chopped, seed-stage timothy hay, molasses, and ground corn for 12 days. The cows were then assigned to four groups; two groups of two, and two groups of three cows each.

The two experimental rations consisted of low-protein timothy hay, molasses, and either concentrate mixture A or B. The composition of the two mixtures is presented in Table 1. Each ration was fed both in the "regular" way and "mixed." The regular way consisted of moistening the hay with molasses diluted 1:1 with water and then placing the concentrate on top of the hay. The hay was treated in the same manner when the mixed ration was fed except that the concentrate was thoroughly mixed into the hay in the manger.

TABLE 1
Ingredients used in the concentrate mixtures

Ingredients	A	B
	(lb.)	*(lb.)*
Yellow corn	1,020	1,200
Soybean oil meal	180	—
Urea[a]	—	25
Salt	12	12
Dicalcium phosphate	24	24
Sodium sulfate	—	7
Total	1,236	1,268

[a] Feed grade.

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A Latin square design was used. Each experimental period consisted of 9 days immediately preceded by a 12-day preliminary period.

The average daily milk production for each 9-day period was determined as fat-corrected milk (FCM) based on 3-day composite butterfat tests. The cows were weighed once every 3 days. The cows were fed twice daily, and all feeds were weighed at the time of feeding. The refused feed was weighed back every third day and its moisture content was determined. Samples of all feeds were taken once each experimental period for chemical analysis. The chemical composition, total digestible nutrient, and digestible protein content of the feeds are presented in Table 2.

TABLE 2

Average chemical composition,[a] TDN, and digestible protein content of the experimental feeds

Feed	Dry matter	Crude protein	Crude fiber	Ether extract	NFE	Ash	S	TDN	DCP[b]
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*
Mixture A	85.9	14.1	2.6	3.14	61.8	4.30	0.18	76.3[c]	11.6[c]
Mixture B	86.5	13.8	2.0	3.42	63.1	4.16	0.24	74.4[c]	6.6[c, d]
Cane molasses	64.0	3.6	—	—	52.6	7.80	0.43	45.8[c]	0.0[c]
Timothy hay	88.0	6.4	30.2	1.77	45.9	3.75	0.13	48.6	1.9

[a] Averages of four samples.
[b] Digestible crude protein.
[c] Calculated from Morrison's *(5)* coefficients of digestibility.
[d] Excluding the urea equivalent.

RESULTS AND DISCUSSION

This experiment was designed to insure protein deficiency during the depletion period by feeding a low-protein ration and then measuring the results in terms of milk production. The low-protein ration reduced the amount of FCM produced approximately 20% during the 12-day period, but milk production leveled off after 6 days and remained approximately at this level for the duration of the experiment. The most striking result obtained during the depletion period was an average loss of 55 lb. in body weight. Some of the loss in weight might be attributed to a decrease in "fill," but this is rather unlikely since about three-fourths of the loss occurred after the cows had received the depletion ration for 6 days. This contention is substantiated further by the fact that body weight was maintained during the next 84 days, during which time the cows received the same basal ration plus a nitrogen supplement.

In the second and fourth periods one cow refused to eat the urea-supplemented ration and had to be removed from the experiment. The cow recovered rapidly when changed to the regular herd ration. The data obtained on this cow were excluded from the results. As this cow was in a 2-cow group, she was replaced by a cow from a 3-cow group that had been subjected to the same ration sequence, but that had been on the other method of feeding. The data obtained on the latter cow during the first period were also excluded from the results, so only 35 cow-periods were analyzed.

Sodium sulfate was incorporated in concentrate mixture B to insure sufficient

sulfur for the efficient utilization of the urea-nitrogen since Thomas *et al. (10)* and Starks *et al. (9)* had previously demonstrated its essentiality. Subsequent analyses showed that all of the feeds, particularly the molasses, contained ample sulfur to supply the needs of the cows without supplementation. Davis *et al. (3)* recently reported that the usual feed mixtures contain sufficient sulfur for the utilization of urea, as previously demonstrated by Lofgreen *et al. (4)* with a lamb ration.

Observations on the eating habits of cows indicate that the natural intelligence of the animals should not be taken lightly. When the ration was fed mixed, the cows shook as much of the concentrate out of the hay as possible and ate it first. This process was repeated until no concentrate was mixed with the hay. Several more minutes were required for the complete consumption of the concentrate portion of the ration when fed mixed than when fed regular.

The analyses of variance of the TDN and digestible protein intakes and the amount of FCM produced are summarized in Table 3. The means and their

TABLE 3

Analyses of variance of TDN and digestible protein intakes, and the amount of FCM produced

Source of variation	Degrees of freedom	Mean squares		
		TDN intake	Digestible protein intake	FCM produced
Groups	3	561[a]	625[b]	218[b]
Periods	3	33	182[b]	5
Treatments	3	14	4,439[b]	3
Urea vs. SBOM	1	38	13,308[b]	6
Reg. vs. mixed	1	3	2	2
Interaction	1	1	6	1
Error	25	155	28	10

[a] Significant at the 5% level of probability.

[b] Significant at the 1% level of probability.

TABLE 4

The means and their standard errors of the per cent of TDN and digestible protein intakes and the pounds of milk produced per day by the various treatments and methods of feeding

Method of feeding	Supplement		
	SBOM	Urea	Combined
TDN intake (per cent of Morrison's maximum requirement)			
Regular	121 ± 4	119 ± 4	120 ± 3
Mixed	120 ± 5	118 ± 5	119 ± 3
Combined	121 ± 3	119 ± 3	120 ± 2
Dig. protein intake[a] (per cent of Morrison's minimum requirement)			
Regular	107 ± 4	67 ± 2	86 ± 5
Mixed	106 ± 4	67 ± 3	86 ± 5
Combined	106 ± 3	67 ± 2	86 ± 3
FCM produced (lb/day)			
Regular	26.8 ± 1.6	28.0 ± 1.6	27.4 ± 1.1
Mixed	27.6 ± 2.0	28.2 ± 2.1	27.9 ± 1.4
Combined	27.2 ± 1.2	28.1 ± 1.5	27.7 ± 0.9

[a] Excluding urea.

standard errors obtained for these three variables, both by supplement and method of feeding, are presented in Table 4. The TDN intake was in considerable excess of Morrison's *(5)* maximum requirement. The digestible protein intake, excluding the protein equivalent of urea, was in excess of Morrison's minimum recommendation when the soybean oil meal was fed, but only 67% of that recommended when urea was fed. The urea supplied 29 ± 1% of the minimum requirement of digestible protein. Three of the cows consumed less than the recommended minimum amount of digestible protein. Under the conditions of this experiment, the amount of FCM produced did not vary significantly, which would indicate that the milk-producing value of the urea supplement was equivalent to that of soybean oil meal. These results are essentially in agreement with those of Owen *et al. (6)*, Rupel *et al. (8)*, Archibald *(1)*, and Willett *et al. (12)*.

As suggested by Thompson *et al.(11)*, the bare maintenance of body weight of the cows receiving 120% of Morrison's *(5)* maximum recommended TDN requirement may have been caused by a depression of the digestibility of the feed nutrients due to the subnormal level of protein in the ration. Bell *et al. (2)*, however, showed that protein (N × 6.25) was the only constituent whose digestibility was increased when urea was added to a low-protein ration. The mean crude protein intake of the cows on this experiment was 8.7 ± 0.5% of the total ration and varied from 2.8 to 3.5 lb. per day. TDN intake was apparently eliminated as a limiting factor because the body weight of the cows was maintained. The excess TDN intake was not utilized efficiently.

SUMMARY

An experiment was conducted with 10 cows over a period of 12 weeks in which each of four treatments was tested by the Latin square design to determine the amount of milk produced by a urea and corn supplement compared to soybean oil meal. In all cases a low-protein roughage was fed, but the supplement was fed either by intimately mixing in the roughage or by placing it on top of the roughage.

No benefit was derived from mixing the supplement in the roughage because the cows promptly separated and consumed the supplement before eating the roughage. One cow consistently refused to eat the experimental ration containing urea.

The maintenance of body weight and the lack of significant difference in milk production as the result of treatment indicated that urea and soybean oil meal were of comparable value under the conditions of this experiment.

ACKNOWLEDGMENTS

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STIMULATION OF LACTIC ACID BACTERIA BY SUBSTANCES CONCOMITANT TO CERTAIN PROTEOLYTIC ENZYMES[1]

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The addition of certain protein hydrolytic products to semi-synthetic and synthetic media has led to observations of enhanced growth and/or metabolic activity of a number of bacterial genera. A classic study in this phase of bacterial nutrition was that of Woolley and his associates, who used the term "strepogenin" to denote the growth-stimulatory effect of various peptides. They observed that trypsinized casein *(8)* and acid hydrolyzed casein *(9)* contained hydrolytic cleavage products (presumably peptides) that markedly increased the growth response of several genera of lactic acid bacteria. Subsequent investigations failed to elucidate the structure of the active peptides in casein hydrolyzates; however, certain synthetic peptides containing glutamic acid exhibited activity, although this activity was much less than that of hydrolyzed casein *(11)*.

Scott *et al. (7)* noted that trypsinized-lactalbumin contained strepogenin activity for *Lactobacillus casei* and confirmed Woolley's observation that trypsinized casein was a good source of strepogenin. They recognized, however, that trypsin contained strepogenin activity and corrected for it in evaluating the strepogenin activity of casein. More recently, Kodicek and Mistry *(4)* reported that a number of highly purified proteins upon partial acid hydrolysis yielded hydrolyzates containing strepogenin activity. Kennedy *(3)* found that commercially prepared lactalbumin hydrolyzate[2] (enzymatic) stimulated the growth of *L. casei* in a medium containing strepogenin. This hydrolyzate also stimulated acid production of *L. casei* and *Streptococcus lactis* when grown in milk.

The activity of several synthetic peptides and hydrolyzates of various proteins prepared in different manners would lead to the conclusion that the strepogenin moiety of various proteins does not exist as a distinct entity with a definite structure; rather, strepogenin might well exist in many structural forms, the growth promoting abilities of which are dependent upon the organism under study and the medium employed. Since enzymatic hydrolyzates of lactalbumin could elicit a response in the presence of adequate levels of pancreatinized casein, it was the purpose of this investigation to study the source and nature of the stimulatory factor(s) in lactalbumin hydrolyzates.

EXPERIMENTAL PROCEDURE

Assay of lactalbumin hydrolyzate in a medium containing strepogenin. Stab stock cultures of *L. casei* (A.T.C.C. 7469) were maintained on riboflavin assay

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[2] Lactalbumin hydrolyzates from Nutritional Biochemicals Corp. and General Biochemicals, Inc.

medium *(5)*. These were transferred monthly and incubated at 35° C. for 24 hours, followed by refrigeration. Inocula for daily use were prepared by transferring a loopful of culture from the stab culture stocks into riboflavin assay medium broth *(5)*. After incubation at 35° C. for 24 hours, the cells were harvested by centrifugation, washed once with sterile 0.85% saline, and recentrifuged. The cells were resuspended in sterile saline to give a suspension having a light transmission of 85% (model 401 Lumetron colorimeter with a 580 filter).

Evaluation of the growth-promoting abilities of lactalbumin hydrolyzate (enzymatic) in the presence of strepogenin derived from casein was accomplished with the medium of Roberts and Snell *(6)*. Double strength medium was dispensed into optically matched 18 × 150 mm. tubes in 5-ml. volumes, covered with aluminum caps, and autoclaved at 112° C. for 6 minutes. All hydrolyzate fractions were adjusted to pH 7.0, sterilized at 112° C. for 6 minutes, then added aseptically to the sterile double strength medium, and final volume of each assay tube was adjusted to 10 ml. with sterile distilled water. Inoculation was accomplished by the addition of 0.05 ml. of the saline cell suspension per tube. The inoculated tubes were incubated at 35° C. for 14 hours. Growth was determined turbidimetrically in the same manner described above for preparing the inocula.

Assay of lactalbumin hydrolyzates in milk. In the assay of lactalbumin hydrolyzates in milk, nonfat dry milk solids were reconstituted with distilled water to give a 9% solids content. By means of a Brewer automatic pipette, 10 ml. volumes of the reconstituted milk were dispensed into 20 × 120 mm. screw-capped vials. The tubes were then sterilized by autoclaving at 15 lb. pressure for 15 minutes.

The assay organisms were *S. lactis* (strain 27) and *L. casei* (A.T.C.C. 7469). Stock cultures were carried in litmus milk and transferred weekly. *L. casei* inocula for daily use were prepared by transfer of 0.3 ml. of the litmus stock into 10 ml. of sterile milk. After incubation at 35° C. for 22-24 hours, the inoculum was diluted 1:10 with sterile skimmilk, and 1 ml. was added to each assay tube. *S. lactis* inoculum for daily use was prepared by transfer of 0.1 ml. of the litmus milk stock culture into 10 ml. of sterile milk. After incubation at 32° C. for 22-24 hours, the culture was diluted 1:10 with sterile milk and 1 ml. was added to each assay tube.

The sterile lactalbumin hydrolyzate fractions were added to the 10 ml. sterile milk assay tubes in such concentrations that the total volume of additions did not exceed 1 ml. After the addition of the hydrolyzate fractions all the assay tubes were adjusted to an 11-ml. volume with sterile distilled water. The assay tubes were incubated at 35° C. for 22 hours when *L. casei* was the assay organism and at 32° C. for 6 hours when *S. lactis* was the assay organism. After incubation, the acid produced in each tube was titrated with *N*/10 NaOH to pH 8.3 with a Fisher Titrimeter.

RESULTS AND DISCUSSION

The preliminary work showed conclusively that the addition of increasing increments of enzymatically hydrolyzed lactalbumin to a medium containing

strepogenin (pancreatic digest of casein) resulted in an increased growth response of *L. casei* (Table 1). A similar response was observed when enzymatically hydrolyzed lactalbumin was added to milk and tested with both *L. casei* and

TABLE 1

Response of L. casei *in Roberts and Snell medium containing added commercial lactalbumin hydrolyzate*[a]

Lactalbumin hydrolyzate solids	Transmission
(mg.)	*(%)*[b]
0	77.8
3	75.8
5	74.8
10	68.3
30	51.5

[a] Purchased from Nutritional Biochemical Corp. and General Biochemicals, Inc.
[b] Represents arithmetic average of four trials.

S. lactis (Figure 1). It was assumed that these responses were due to some cleavage product(s) which resulted from the enzyme attack upon the protein. Since this product(s) elicited a response over and above the growth promotional abilities of strepogenin, it was further assumed that the active cleavage product(s) differed distinctly from the strepogenin moiety of casein.

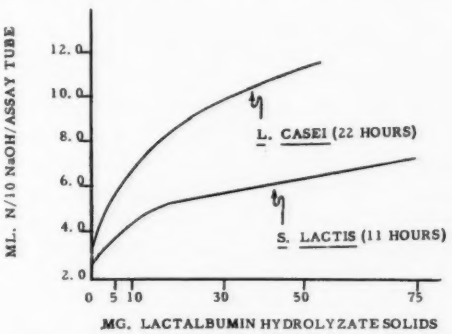

FIG. 1. Response of *L. casei* and *S. lactis* in milk containing added lactalbumin hydrolyzate.

Since it appeared that the active factor(s) arose from enzymatic hydrolysis of lactalbumin, the production of the factor should conform to an enzyme-time relationship. Accordingly, commercial lactalbumin was suspended in water to give a final concentration of 10% by volume. The pH was adjusted to 7.4, and 1-300 trypsin[3] was added at the rate of 0.5%. Toluene and chloroform were added as preservatives, and the mixture was incubated at 35° C. for 24 hours. Aliquots were removed at 0, 2, 4, 8, 16, and 24 hours, respectively, and the

[3] Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 2

Response of L. casei *to lactalbumin hydrolyzed for varying times with trypsin*[a]

Length of hydrolysis	Nitrogen increase	*L. casei* response in	
		Milk	Roberts & Snell medium
(hr.)	*(mg.)*	*(ml. N/10 NaOH/tube)*	*(% transmission)*
Control (no hydrolyzate)	—	5.8	82.5
0	—	9.6	45.0
2	10	10.2	44.5
4	24	10.1	44.5
8	20	10.1	40.0
16	32	10.3	42.0
24	48	10.3	39.0

[a] Nutritional Biochemical Corp. and General Biochemicals, Inc.

enzyme was inactivated by exposure to a temperature of 100° C. for a period of 10 minutes. After the mixture was filtered and the pH adjusted to 7.0, it was sterilized and added aseptically to milk assay tubes and to Roberts and Snell assay tubes (Table 2). In both the milk assay and the semi-synthetic medium assay no appreciable increase in activity could be detected over the zero hour reading. Formol titration values, however, showed that extensive hydrolysis of the lactalbumin had occurred.

At this point it appeared that the active factor(s) was not associated with the tryptic cleavage products but rather was concomitant with either the intact protein or the enzyme. In order to ascertain which moiety possessed the activity, lactalbumin and heat-inactivated trypsin were added singly to both the semi-synthetic medium and the milk. Lactalbumin additions to the semi-synthetic medium manifested no increased growth response; a slight response was observed upon its addition to milk. Additions of inactivated enzyme to either the semi-synthetic medium or to milk resulted in a stimulation comparable to that observed upon the addition of lactalbumin hydrolyzates containing an equivalent amount of 1-300 trypsin (Table 3). Further evaluation of this activity showed

TABLE 3

Response of L. casei *and* S. lactis *in milk supplemented with inactivated trypsin and pancreatin*[a] *(aqueous solutions)*

Enzyme	Ml.*N*/10 NaOH required to titrate each assay tube			
	L. casei		*S. lactis*	
	Trypsin	Pancreatin	Trypsin	Pancreatin
(mg.)				
0	4.1	4.1	2.3	2.3
10	7.3	5.7	3.9	3.2
20	7.8	6.5	4.3	3.9
30	9.4	7.1	—	4.6
50	10.5	8.1	5.5	5.1

[a] Pancreatin and 1-300 trypsin obtained from Nutritional Biochemicals Corp.

TABLE 4

Response of L. casei *to inactivated enzymes (aqueous solutions) when tested in the medium of Roberts and Snell*

Enzyme	Mg. assayed	Transmission (%)[a]
0	0	85.5
2X trypsin	2	83.0
"	4	84.5
pancreatin	2	75.0
"	4	73.5
1-300 trypsin	2	74.5
"	4	72.5

[a] Average.

that it was present in pancreatin as well as partially purified trypsin but was not present in crystalline trypsin (Worthington 2X).

These results demonstrated that the stimulatory activity of trypsinized lactalbumin was not associated with the cleavage products of lactalbumin when tested either in a semi-synthetic medium which contained strepogenin or in milk. The activity appeared to reside with the partially purified enzyme. Crystallization of the enzyme resulted in a complete loss of stimulation, thus indicating that the active factor(s) was not associated with the enzyme but rather resided in the impurities accompanying the enzyme.

In light of the foregoing, it appeared advisable to investigate trypsinized casein to ascertain the source(s) of the active factor(s) (Figure 2). Accordingly,

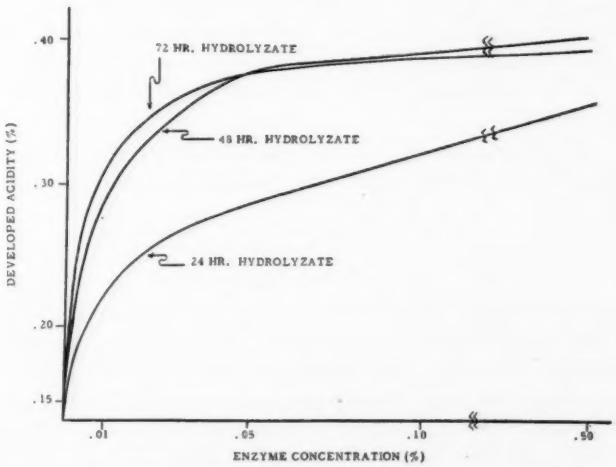

FIG. 2. The effect of enzyme (1:300 trypsin) level on the response of *S. lactis* to trypsinized casein added to milk.

a 10% casein suspension was hydrolyzed by the addition of 1-300 trypsin in concentrations of 0.01, 0.05, 0.10, and 0.50%. The activity of these hydrolyzates was checked at 24, 48, and 72 hours, respectively, by assaying 0.1 ml. of the digests in milk with *S. lactis* as the test organism. The data showed that within each time period the activity of the hydrolyzates increased directly with the level of enzyme used; increased hydrolysis time, however, did tend to eliminate an increased response due to additions of increasing increments to enzyme. This indicated that the activity arose from casein cleavage products as well as from the enzyme. These data appeared to be in agreement with the observations of Scott *et al. (7)*. Subsequent investigation showed that partially purified pepsin, takadiastase, steapsin, erepsin, and papain all contained similar stimulatory activities.

Finding that enzyme preparations contained growth-stimulatory factors would indicate need for a reconsideration of our knowledge of the sources of strepogenin. The present inadequate information on the composition of strepogenin may be the result of the existence of multiple peptides of varying degrees of activity in enzymatic digests of proteins. With only a few exceptions (Scott *et al., 7* and Welsch and Thibaut, *10)*, measurements of strepogenin activity have included factors contained in the enzyme preparations as well as active cleavage products from the hydrolyzed protein. The use of crystalline enzymes would be clearly indicated for the study of stimulatory components arising from the protein.

Application of these substances to various milk fermentations has been limited, although it would seem that these and similar substances hold considerable promise in accelerating the ripening of various milk products. Anderson and Elliker *(1, 2)* have observed that protein hydrolyzates and certain liver fractions accelerated and/or increased the growth response of the lactic group of streptococci in synthetic media and in milk. It is possible that various tissues from which enzymes are obtained or residues from the purification of certain enzymes may prove to be more satisfactory sources of these factors than hydrolytic products of proteins.

Studies are in progress on the characterization of the growth-promoting factors associated with various tissues and residues from the purification of certain enzymes.

SUMMARY

Enzymatic hydrolyzate of lactalbumin possessed growth-stimulatory ability for certain lactic acid bacteria when added to milk and to a nutritionally complete semi-synthetic medium. The stimulation afforded by this hydrolyzate has been shown to originate in the enzyme preparation used. The enzyme itself was not stimulatory, as evidenced by the fact that impure trypsin preparations were stimulatory but crystalline trypsin was not. The stimulatory factor(s) associated with impure preparations of certain enzymes must be considered in any study of "strepogenin" activity of protein hydrolyzates prepared with such enzymes.

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THE EFFECT OF SODIUM ACETATE FEEDING ON MILK AND FAT YIELD, BLOOD SUGAR, AND BLOOD KETONES OF DAIRY COWS[1,2,3]

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It has been shown that a decrease in the level of acetic acid in the rumen contents is associated with a marked depression of milk fat in cows fed a high-concentrate diet with either very limited amounts of long hay or with very liberal amounts of alfalfa meal as the sole roughage *(8, 9)*. In either case, the feeding of sodium acetate caused a recovery toward normal milk fat levels, indicating that the acetates which are normally a product of rumen fermentation have an essential role in the synthesis of milk fat.

A study of the value of sodium acetate feeding for treatment of ketosis has been reported *(5)*. In most cows which recovered after the feeding of sodium acetate, blood sugar increased and blood ketones decreased. However, since under the conditions of the experiment it was not possible to set up an untreated control group, recovery may have been spontaneous rather than specifically due to acetate feeding. Recovery from ketosis is ordinarily accompanied by a rise in blood sugar and a decrease in blood ketones, and the changes in these blood values may have been incidental to the recovery rather than a specific effect of the sodium acetate feeding. Schultz and Smith *(7)* observed a slight increase in blood sugar and no appreciable change in blood ketones after the feeding of acetic acid to goats.

The stimulating effect of acetate feeding to cows with diet-depressed milk fat and rumen acetate raises a question as to the effect upon cows with normal milk fat and rumen acetate levels. Should a comparable increase in milk fat percentage result, it would have practical implications in the production-testing of dairy cows if sodium acetate were fed deliberately as a milk fat stimulant, or if indicated in the treatment of ketosis.

This experiment was designed to determine the effect of sodium acetate, when fed to cows in normal health and on a normal roughage diet, on milk and fat production, milk fat percentage, blood glucose and blood ketones.

EXPERIMENTAL PROCEDURE

A double reversal design was chosen by which it would be possible to detect small differences in blood and milk values resulting from the feeding of sodium

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acetate *(3)*. In each of the three 10-day periods, the first 4 days were used as a transition period and the last 6 days for the comparison. Eighteen cows of four breeds were paired according to breed, stage of lactation, location in barn, age, milk production, and fat test. Cows of each pair were then assigned at random to Group A or B.

TABLE 1

The effect of feeding 1 lb. of sodium acetate daily on milk and fat production, milk fat test, blood glucose, and blood ketones of 18 cows

	Average fat test	Average fat/day	Average milk/day	Blood glucose	Blood ketones
	(%)	*(lb.)*	*(lb.)*	*(mg. %)*	*(mg. %)*
Control	4.743	1.346	28.52	58.056	2.772
Sodium acetate	4.822	1.360	28.39	58.092	2.742
Difference	0.079	0.014	−0.13	0.036	−0.030
LSD (0.05) [a]	0.102	0.065	0.84	0.609	0.183

[a] LSD (0.05) = least significant difference at 0.05 level.

The concentrate mixture, fed at the level of 16 lb. per day to all cows, consisted of 700 lb. each of ground oats and corn, 400 lb. of wheat bran, 200 lb. of linseed or soybean oil meal, 20 lb. of steamed bone meal, 20 lb. of trace mineralized salt, and ½ lb. of irradiated yeast. During the period of sodium acetate feeding, each cow received 1 lb. of technical grade sodium acetate daily, incorporated in the concentrate mixture. In a number of instances individual cows at one or more feedings refused to eat the grain containing sodium acetate. When this occurred the sodium acetate was given separately in gelatin capsules. The cows were given a liberal feeding of corn silage in the evenings and allowed free access to alfalfa-grass hay during the day.

Butterfat tests were made of each individual milking by the Babcock method. Blood samples were taken from the jugular vein on the first and fifth days of each 10-day period. Potassium oxalate and sodium fluoride were used as anticoagulant and preservative, respectively *(3)*. Protein-free filtrates of the whole blood were prepared by the Folin-Wu method *(4)*. Blood ketones and glucose were determined by methods outlined by Behre *et al. (1)* and Nelson *(6)*, respectively.

Total milk and fat production for the 6-day periods was determined for each cow by adding the daily milk and fat weights. The average fat test was then calculated. The values for blood glucose and ketones were determined by averaging the values obtained for the samples taken on the fifth and tenth days.

RESULTS AND DISCUSSION

There were no significant differences in milk fat test, total milk or fat production, blood glucose, or blood ketones between the cows receiving the control ration and those receiving 1 lb. of sodium acetate daily (Table 1). The sensitivity of the experiment, as indicated by the least significant differences, was sufficient to detect any differences of importance.

It seems that there is a minimum level of acetate required for production of milk of normal fat content and that feeding of acetate up to this amount will cause recovery from the diet-depressed milk fat, but additional amounts above this minimum level will not cause further increases. The feeding of sodium acetate to cows on production test should not contribute to inaccuracy of the records.

SUMMARY

The effects of feeding 1 lb. of sodium acetate daily for 10-day periods on milk and fat production, milk fat test, blood glucose, and blood ketones of normal cows receiving normal diets were determined. There were no significant differences ($P = 0.05$), in any of the values studied, between the cows receiving acetate and the controls. The sensitivity of the experiment was sufficient to detect average differences of 0.1% in fat test, 0.06 lb. of fat, or 0.8 lb. of milk per day, 0.6 mg. % blood glucose or 0.18 mg. % blood ketones.

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TECHNICAL NOTES

IDENTIFICATION OF α-LACTALBUMIN IN THE ELECTROPHORETIC PATTERN OF MILK SERUM PROTEINS[1]

The protein α-lactalbumin recently has been isolated from milk serum and characterized by Gordon and Semmett *(2)*, who identified it with the *a* component of the ultracentrifugal patterns of Pedersen *(5)* and also with the "crystalline insoluble substance" described by Sørenson and Sørensen *(8)*. Gordon and Semmett reported an electrophoretic mobility for isolated α-lactalbumin of -4.2[2] (veronal buffer, pH 8.5, ionic strength = 0.1, temp. = 0.5° C.), and they suggested that it is identical with the component observed by Smith *(7)* in an electrophoretic analysis of the milk serum proteins having a mobility of -4.5 (veronal buffer, pH 8.6, ionic strength = 0.1, temp. = 1° C.). In Smith's electrophoretic pattern this component was the fourth of six in order of increasing mobility. It appeared as an asymmetry on the peak representing β-lactoglobulin and comprised about 12% of the total proteins. The third of Smith's six components (Component III) was represented by a distinct peak having a mobility of -3.6 and constituted about 18% of the total protein. Actually the mobilities and percentages reported by Smith varied with protein concentration. The values given here are for a solution containing 1.23% protein.

In a recent review of the milk proteins, McMeekin *(4)* has reiterated Gordon and Semmett's identification of α-lactalbumin with Smith's Component IV and furthermore has stated (page 412) that "the globulin with a mobility of $\mu = -3.6$, which amounts to 18 per cent of the protein of whey, has not been obtained in pure form."

It is of utmost importance in interpreting changes in electrophoretic patterns produced by various treatments to know which component constitutes each peak. Since the identification of α-lactalbumin with the electrophoretic Component IV is based merely on a comparison of mobilities obtained in two different laboratories, we have sought to establish the identification more unequivocally by the following direct experiment.

Concentrated solutions of the milk serum proteins were prepared in 0.12 *M* veronal buffer, pH 8.6, ionic strength 0.1, with and without the addition of twice crystallized α-lactalbumin. These were dialyzed against the same buffer and the electrophoretic patterns determined. Each author performed the experiment independently in his own laboratory with different protein preparations. Figure 1 and Table 1 give data obtained at the University of Illinois

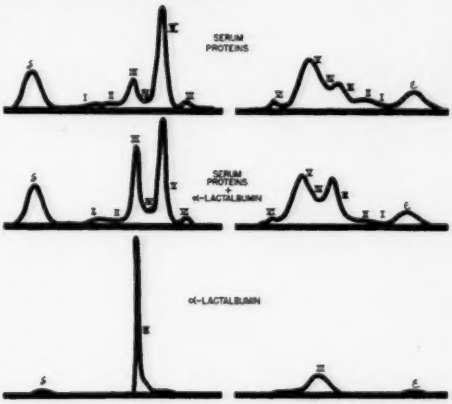

FIG. 1. Electrophoretic comparison of the milk serum proteins, the milk serum proteins plus added α-lactalbumin, and α-lactalbumin. Protein concentrations were, respectively, 2.03, 2.43, and 0.65%. Time = 5,580 sec., temp. = 1.2° C., field strength = 7.8 volts/cm.[2]

with an AMINCO apparatus and show that the added α-lactabumin quantitatively enhanced the peak corresponding to Smith's Component III with a mobility of -3.6. Comparable results were obtained at the University of Minnesota with a Klett apparatus and with protein concentrations of 1.31 and 1.47%, time of 10,800 seconds, temperature of 4° C., and field strength of 6.00 volts/cm.[2] In this experiment analysis of the patterns revealed that there was 22% Component III and 62% Components IV plus V in the original serum protein, and this was changed to 30% and 58%, respectively, in the sample enriched with α-lactalbumin. Thus, the amount of α-lactalbumin added (0.16 g/100 ml.) appeared quantitatively in the peak representing Component III [$(1.47 \times 0.30) - (1.31 \times 0.22) = 0.15$ g/100 ml.]. The mobilities of Components III and IV were unchanged by adding α-lactalbumin, although they were slightly higher than those reported in Table 1 because of the higher temperatures used. Thus, there is little doubt that the peak designated Component III represents α-lactalbumin in the original serum protein mixture and that Smith's Component IV is the one which has as yet not been identified.

Table 1 also shows the mobility of twice crystallized α-lactalbumin run separately under the same electrophoretic conditions as when mixed with the milk serum proteins. It should be noted that the descending mobility for α-lactalbumin (-4.4) is more rapid in purified form than when the other serum proteins are present (-3.6) and that this mobility corresponds closely to that reported by Smith (-4.5) for Com-

[1] Joint contribution from the Illinois and Minnesota Agricultural Experiment Stations. Paper No. 3317. Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] All mobilities are expressed as $\times 10^{-5}$ cm.^{2} volt^{-1} sec.$^{-1}$

TABLE 1

Electrophoretic analysis of the serum proteins and α-lactalbumin

Designation by Smith				Serum proteins				Serum proteins plus α-lactalbumin				α-lactalbumin			
				Mobility				Mobility				Mobility			
Component	No.	Mobility	Per cent of total	Asc.[a]	Desc.	Per cent of total	Conc.	Asc.[a]	Desc.	Per cent of total	Conc.	Asc.[a]	Desc.	Per cent of total	Conc.
							(g/100 ml.)				*(g/100 ml.)*				*(g/100 ml.)*
Immune euglobulin	I	−1.7	6	−1.9	−1.7	10	0.19	−1.9	−1.7	8	0.19				
pseudoglobulin	II	−2.5	4	−2.8	−2.6	5	0.11	−2.7	−2.4	5	0.12				
Component	III	−3.6	18	−3.8	−3.6	21	0.42	−3.9	−3.6	32	0.76	−3.9	−4.4	81	0.53
Component	IV	−4.5	12	−4.6	−4.3	3	0.07	−4.6	−4.3	3	0.07				
β-Lactoglobulin	V	−5.1	55	−5.2	−5.0	53	1.07	−5.2	−5.0	45	1.09			19[b]	0.12
Component[c]	VI	−6.4	5	−6.5	−6.5	6	0.12	−6.4	−6.4	5	0.12				
Component[d]				−7.6	−7.5	2	0.05	−7.6	−7.2	2	0.04				

[a] Apparent mobilities (uncorrected for conductivity difference in ascending limb).

[b] Other 19% accounted for by materials with mobilities of Components IV, V, and VI (see Fig. 1).

[c] Isolated and characterized by Polis *et al. (6)* and Coulson and Stevens *(1)* as being identical with blood serum albumin.

[d] This material with a mobility greater than serum albumin in this buffer system was not included by Smith but has been repeatedly noted and is included in the calculations.

ponent IV. This fact probably accounts for the suggestion of Gordon and Semmett that α-lactalbumin and Component IV are identical. Although no change in the ascending mobility for the purified α-lactalbumin was found, a peculiar one-sided pattern was obtained (see Figure 1). The reasons for this are unknown at present; however, the same pattern was obtained in each author's laboratory with different electrophoretic instruments and with two samples of twice crystallized α-lactalbumin.[3]

With this placing of α-lactalbumin in electrophoretic patterns of the milk serum proteins, over 80% of the proteins now have been identified. Further studies on the identity of Smith's Component IV and other minor components evident in the electrophoretic patterns of milk serum will be published shortly *(3)*.

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[3] One sample was supplied by W. G. Gordon of the Eastern Regional Labs., USDA.

LIPASE ACTIVITY IN FRESH MILK AS RELATED TO PORTIONS OF MILK DRAWN AND FAT GLOBULE SIZE[1,2]

The results of a study in which different portions of milk drawn were subjected to analysis for free fatty acids has been reported previously *(3)*. The results revealed that, in general, an indirect relationship existed between the free fatty acid content and the portion of milk drawn at milking, i.e., the first portion was highest in free fatty acid content and the last portion (the strippings) was lowest. This appeared to be related also to a lower fat content in the first portion of milk.

The purpose of this study was to survey the relationship between the fat globule size, the free fatty acid content, and lipase activity of the various portions of freshly drawn milk.

Three portions of milk were drawn from individual cows, the first 500 ml. of milk being designated as Portion A; the second aliquot sample, which represented about 85% of the total milk yield, was designated as Portion B; and the third aliquot, which represented the strippings, was called Portion C. The various portions were cooled immediately to 5° C. The procedures used in sampling and analysis were the same as previously reported *(3)*.

To determine the lipolytic activity in the various portions of milk, the samples were incubated at 5° C. for 96 hours. Acid degree measurements were made at the beginning and at the end of the incubation period, and lipase activity was reported as the increase in acid degree values.

Fat globule size of Portions A and C was determined by microscopic examination with the Nile Blue sulfate staining techniques of Cole and Smith *(1)* as modified by Loo *et al. (2)*. From 90 to 100 globules were measured in each determination, which usually required the examination of 20 to 30 fields. The arithmetical average diameter of fat globules (in microns) in the sample was calculated.

Milk from 16 cows in various stages of lactation representing five breeds was studied. The samples were examined for lipolytic organisms by using nutrient milk agar plates, which were incubated 48 hours at 37° C. and then flooded with Nile Blue sulfate after incubation. None of the samples analyzed showed the presence of lipolytic organisms.

Milk was obtained from four cows early in lactation (20-40 days), four cows in middle lactation (100-190 days), and eight cows in late lactation (290-355 days). The data obtained are presented as averages, with the variability in the results being shown by the ranges of values obtained.

[1] Technical Paper 3-54. Department of Food Technology, Institute of Nutrition and Food Technology, The Ohio State University.

[2] Supported in part by funds provided by the Research and Marketing Act of 1946 through the Bureau of Dairy Industry, U.S.D.A., and by the Ohio Dairy Products Research Fund.

TABLE 1

Relationship of fat globule size, stage of lactation, and portion of milk to the initial free fatty acid content and lipase activity of fresh milk

No. of cows	Stage of lactation	Portion of milk	Size of milkfat globules		Fat content		Lipase activity[a]	
			Range	Average	Range	Average	Range	Average
4	Early (20-40 days)	A	1.69-2.08	1.91	1.47-1.63	1.55	0.25-2.89	1.40
		B	—	—	0.45-0.60	0.51	0.13-1.59	0.72
		C	2.84-3.16	3.10	0.50-0.57	0.54	0.20-0.97	0.52
4	Middle (100-190 days)	A	0.94-1.46	1.30	0.99-2.27	1.86	1.13-3.78	2.03
		B	—	—	0.59-0.84	0.72	0.47-1.62	0.99
		C	2.12-2.42	2.34	0.40-0.87	0.66	0.36-1.16	0.64
8	Late (230-360 days)	A	1.48-1.87	1.66	0.56-2.80	1.16	0.27-3.78	1.54
		B	—	—	0.59-1.42	0.86	0.21-2.48	1.16
		C	2.05-2.67	2.38	0.40-1.52	0.84	0.09-3.17	0.93

[a] Expressed as the increase in acid degree values between 0 and 96 hours.

Data which reveal the relationship of the stage of lactation, portion of milkfat content, fat globule size, the initial free fatty content, and lipase activity are presented in Table 1. Normally active lipase (active on cooling milk) was observed in the milk of all 16 cows and in all portions of milk drawn. Regardless of the stage of lactation, the data reveal that the smaller fat globule size is related inversely to the higher initial free fatty acid content and higher lipase activity. As previously reported *(3)*, the highest initial free fatty acid content was found in the Portion A, with the two other portions exhibiting little difference in acid degree values. The fat content was lower in Portion A, and the fat globule size was also smallest in the first milk drawn. The fat globules ranged in size from 0.94 to 2.08 μ in Portion A and from 2.05 to 3.16 μ in Portion C.

Similarly, lipase activity, which is expressed as the increase in acid degree values during the 96 hours of incubation at 4° C. was highest in the first portion of milk drawn. This relationship was exhibited in the milk of 15 of the 16 cows studied. In contrast, the milk from the stripping revealed the least lipase activity for 14 of the 16 cows.

The milk with the greatest lipase activity possessed smaller fat globules. However, milk with small globules did not always possess high activity. The milk with an average fat globule size of 2.0 μ or less had a lipase activity ranging from 0.23 to 3.77, with an average of 2.11. The milk with an average fat globule size of more than 2.0 μ had a lipase activity of 0.20 to 1.80, with an average of 0.58. The inconsistencies in the inverse relationship between fat globule size and lipase activity may be exceptions to the general trend.

and the initial free fatty acid content and lipase activity of the milk. However, further study is necessary to determine whether or not this is a cause and effect relationship.

The limited data indicate an inverse interrelationship between the average fat globule size

The observations in this study suggest, but do not purport to prove, several interesting possibilities in regard to milk lipase: *(a)* all milk may contain normally active lipase, which is measurable by the use of more sensitive methods; *(b)* lipase activity may take place in the udder, as evidenced by higher free fatty acid content in first milk drawn; *(c)* the concentration of normally active lipase varies with the individuality of the cow, and this activity is not necessarily related to the period of lactation.

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REICHERT-MEISSL VALUE: ITS IMPORTANCE AND LIMITATIONS IN THE DETECTION OF FOREIGN FATS IN BUTTERFAT

Consideration is being given by some state regulatory agencies to the use of Reichert-Meissl value for the detection of foreign fats in butterfat. Before this test is adopted, it may be well to consider what this value measures and what factors affect it. The Reichert-Meissl value gives a measure of the volatile water-soluble fatty acids (butyric acid and some of caproic acid). However, this value as measured by the official method does not represent the entire amount of volatile fatty acids present in the fat and is only comparative; therefore, strict adherence to the dimensions of apparatus and methods of analysis is necessary in order to obtain comparable results. In spite of these limitations, this analytical constant is most useful in determining the purity of butterfat. Butterfat contains high proportions of volatile fatty acids, whereas vegetable and other animal fats contain very little or no volatile fatty acids.

Variations in the Reichert-Meissl Value

A wide variation in the Reichert-Meissl value of butterfat may be expected, since the composition of butterfat is affected by factors such as breed, age of the cow, stage of lactation, individuality, season, the nutritional condition of the animal, and the nature of the feed.

Breed. The average Reichert-Meissl values of butterfat from different breeds of cows show wide variations: Ayrshire, 30.3; Shorthorn, 21.0; Jersey, 26.7-33.63; Holstein, 25.5-28.0.

Stage of lactation. The butterfat secreted early in lactation has a high Reichert-Meissl value, which drops to a fairly constant value by the third month. Late in the lactation, a further decline is noted. In all, a decrease of about six units in the Reichert-Meissl value during the 9 months of lactation period is observed.

Feed. The feed is the most important factor that affects the composition of butterfat. The amount of carbohydrate, fat, and protein present in the ration has a definite effect on the characteristics of butterfat. Pasture feeding of both grasses and legumes decreases Reichert-Meissl value. However, green fodder preserved as ensilage has less effect than the original green fodder. Corn silage increases Reichert-Meissl value. The type of fat present in the concentrate has the greatest influence on the composition of butterfat. When cows are fed cottonseed whole meal, the Reichert-Meissl value decreases, the effect being most marked when the meal is fed with dried hay as supplement; on such feeding, butterfat with a Reichert-Meissl value as low as 18-23 has been obtained. The effect of coconut oil feeding on Reichert-Meissl value has been found to vary from a slight decrease to no change. Soyabean oil, corn oil, linseed oil, and peanut oil decrease Reichert-Meissl value. With corn oil, a Reichert-Meissl value as low as 14.4 has been obtained.

Age. Limited data available in India show that young cows secrete butterfat with higher Reichert-Meissl values than do older cows.

Season. Since each season is associated with certain weather conditions and diet, the composition of butterfat might be expected to vary. Figure 1 illustrates the changes in the average

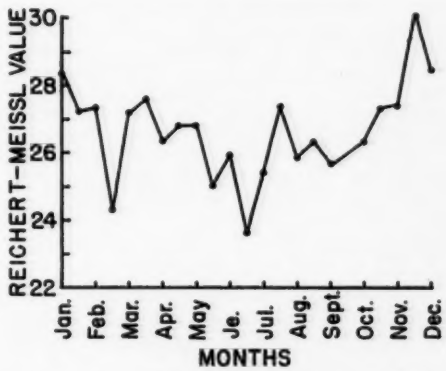

FIG. 1. Seasonal variation in the average Reichert-Meissl value of butterfat of herd milk (approximately 300 cows) from different breeds. (Sindhi, Gir, Crossbred).

Reichert-Meissl value of butterfat from milk of different breeds of cows under temperate weather conditions. The transition of cows from pasture to stall feeding increases the Reichert-Meissl value, whereas changing from indoor winter feed to pasture decreases Reichert-Meissl value. These changes occur within a rather short time. Changes in the environmental temperature between 40 and 90° F. do not affect the Reichert-Meissl value, but temperatures between 90 and 95° F. decrease the Reichert-Meissl value.

Degree of nourishment. Submaintenance feeding and feeding below the producing capacity of the cow lower the Reichert-Meissl value. Inanition decreases markedly the Reichert-Meissl value as much as seven units in 24 hours and 11 units in 48 hours.

Disease. The composition of butterfat is affected by several diseases of the animal. Mastitis, which is one of the commonest infections, causes a decrease in Reichert-Meissl value to a considerable extent.

Effect of Adulteration of Butterfat

Vegetable fats and animal fats or both together may be used as adulterants. The vegetable fat may contain a single fat or mixtures of fats in the form of shortenings. Since the

Reichert-Meissl value of vegetable oils is so very low compared to the butterfat, it would seem that it should be simple enough to detect their presence in butterfat, even in small amounts. However, the Reichert-Meissl value of butterfat is so variable (22.5-30.5) that the presence of foreign fat, even up to 25-30%, will not be detectable on the basis of Reichert-Meissl value alone.

The animal fats used may be beef fat or rearranged lard. Beef fat does not have any Reichert-Meissl value and, therefore, its presence in butterfat has the same effect on the value as the vegetable fat. "Rearranged" lard may be explained as follows: Fats and oils contain mixtures of triglycerides of fatty acids in different proportions. A triglyceride molecule consists of three fatty acids attached to one molecule of glycerol. The process by which the percentage of any one type of triglyceride is changed into another type is known as rearrangement. In recent years, rearranged lard is being manufactured to contain butyric acid up to 10%. If it is used as an adulterant, the detection of foreign fat becomes even harder on the basis of Reichert-Meissl value.

Methods for Fat Extraction

Various methods are available for the extraction of fat from the milk product. The reagents used in different methods vary, and the resulting fat extracted might be affected to a certain extent. For example, the Mojonnier method removes phospholipides, whereas the Minnesota reagent method does not. Consequently, the Reichert-Meissl value may differ slightly from one extraction method to the other.

Conclusions

Since various factors affect the Reichert-Meissl value, and in view of the fact that fats may be rearranged to contain butyric acid, it is impractical to judge the purity of butterfat on Reichert-Meissl value alone. The standards fixed to characterize a given dairy product, such as ice cream, become meaningless unless other factors and analytical constants which bear relationship with the Reichert-Meissl value are taken into consideration.

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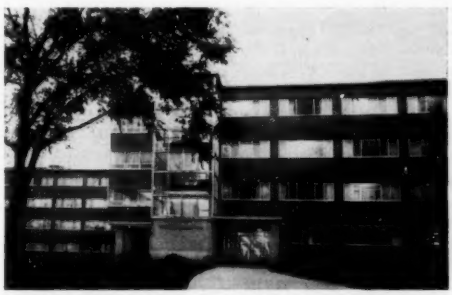
PEOPLE and EVENTS
in the Dairy Science World

Plans Being Made for A.D.S.A. Meeting

Delegates to the annual meeting of the American Dairy Science Assoc., June 20-23, will be housed at Michigan State College, East Lansing, Mich., in three new dormitories which form a series of residence halls. The rooms, lounges, and other facilities are modern and attractively decorated. A large number of members and their families are expected to attend.

Some of the sessions will be held in Kellogg Center at East Lansing. It will be impossible to use large lantern slides or movies in these sessions. Speakers may use 2″ × 2″ slides. Speakers are urged to place all visual aid material in mimeographed form to distribute to those attending the sessions in Kellogg Center.



One of the residence halls at Michigan State College.

Michigan Scientists Find Interesting Results Regarding Soil Fertility and Nutrition

The results of a 10-year study of the relation of soil fertility to the nutritive value of the crops have been recently announced at Michigan State Agricultural College. A team of research workers[1] from five different departments have reported conclusions to the effect that feeds grown on depleted soils do not affect the composition or nutritive value of cow's milk. The research was supported in part by grants from the National Dairy Council on behalf of ADA.

[1] Michigan State College staff members, working under the leadership of L. M. Turk, director of the Michigan Agricultural Experiment Station, included: Farm Crops Dept., S. T. Dexter; Soil Science Dept., Ray Cook; Dept. of Agricultural Chemistry, C. W. Duncan; Dairy Dept., G. M. Ward and Earl Weaver; Foods and Nutrition Dept., Dena C. Cedarquist and Margaret A. Ohlson.

The Study Plan

Eighteen 10-acre fields were laid out on a 210-acre farm with badly run-down soil. The same crops were grown on the two areas—winter wheat, oats, soybeans, corn, and grass hay. In 10 years, over 5,000 lb. of 0-12-12 fertilizer were added to one area, with a top covering of nitrogen on all crops except soybeans. On the other, no mineral fertilizer was added, but nitrogen was used to get crops large enough to harvest. Even with nitrogen treatment, the crops on the depleted soil were sparse. The variety of crops which these acres would sustain was limited.

Two herds of cattle, composed of pairs of half sisters, one half sister to each herd, were fed grain and forage in dry lot. The herds were fed the same way, both getting hay and grain. Heifer calves were saved to get several generations of animals raised on feed from either depleted or fertilized land.

Regular chemical analyses of the crops, as they were fed, and of the milk and blood of the cattle were made. Records of feed eaten, milk produced, breeding and reproduction, and rate of gain were kept on both herds. Samples of milk were taken regularly and fed to colonies of rats to determine the actual feeding value of milk.

Some of the Conclusions about the Milk

1. The consuming public can be assured that milk produced by cows that subsist on feeds produced on depleted land is equal in nutritive value to that of cows fed from fertilized land. If cows' ration is low in protein or minerals, milk production will be decreased, but composition of milk will remain the same.

2. Milk produced by both groups contained as much or more fat, protein, and mineral constituents as are commonly found in market milk.

3. The amount and kind of amino acids in the milk proteins were similar, regardless of whether the feeds were grown on fertilized or unfertilized soil.

4. Vitamin content of the milk produced showed no differences attributable to the feeds. Low levels of carotene and vitamin A were evident. However, this was due to the fact that none of the cows had access to pasture. The values were similar for both herds.

Some of the Conclusions about the Crops

1. Badly depleted soil produces feeds that are just as nutritious as the same kind of feeds grown on fertilized soil. The assumption that commercial fertilizers either increase or decrease the nutritive properties of crops was not substantiated.

2. There is little difference in the chemical composition of cereal grains that can be attributed to fertilization when these grains have grown to maturity.

3. The composition of forage crops that were harvested for hay was found to vary with the type of plant species and stage of maturity in response to fertilization.

4. Seasonal differences appeared to have far more influence on the composition of crops than fertilizer treatment.

With soil from the field experiment, a greenhouse study was set up to determine the effect of lime and fertilizers on the composition of soybeans, oats, and alfalfa. Dry tissue was analyzed for protein, phosphorus, calcium, fiber, ash, and iron. Results showed that lime and fertilizer did not affect all plants in the same manner. Whether or not the treatments improved the crop as animal feed would be determined by species of plant, the kind of livestock to be fed, and the nature of any supplementary feed. It is probably not possible to conclude that any certain fertilizer will always improve the feeding quality of a groups of crops, or even of one certain crop.

Some Conclusions about the Cows

The study also explored the effects of the feed on health, growth, breeding efficiency, feed intake, and milk yields of the cows. Reproduction of animals was not affected by soil fertility according to research results. Birth weights of the two breeds of cattle used for the test groups were not significantly different, except for bull calves of one of the breeds, which averaged 11 lb. heavier in the "unfertilized group." Breeding efficiency was not significantly different, no importance difference was found in growth of heifers, and blood pictures were similar for the cows in the two groups.

A significant difference in milk production by the cows of one of the breeds in favor of the group on unfertilized feeds indicated greater appetite for, and intake of, unfertilized feeds rather than the ability of the feeds to support milk production. No important difference in production of the cows of the other breeds was observed.

Dairy Council Honors Top National Leaders

Honors went to some of the country's top leaders in research, education, human nutrition, and agriculture at the 40th Anniversary Annual Meeting of the National Dairy Council in Chicago, Jan. 24-26.

Among those cited for distinguished contributions to the nutrition field were former PRESIDENT HERBERT C. HOOVER; E. V. MCCOLLUM, emeritus professor of Johns Hopkins Univ.; H. C. HUNT, formerly superintendent of Chicago public schools and now Eliot Professor of Education at Harvard Graduate School of Education; HAZEL K. STIEBELING, director of human nutrition and home economics research, USDA; THOMAS PARRAN, dean, Graduate School of Public Health, Univ. of Pittsburgh; and MRS. NEWTON P. LEONARD, president, National Congress of Parents and Teachers, Chicago. Secretary of Agriculture EZRA TAFT BENSON received a citation prior to addressing the group at the final luncheon on Wednesday.

Hoover was unable to attend the banquet. His award, which cited his work as War Food Administrator in World War I and which "decreed that American families should not be deprived of the nutritional benefits of dairy foods in wartime," was received by W. A. WENTWORTH, member of the NDC board of directors and director of industry relations, The Borden Company, N. Y.

A man who was among the first members at the founding of the National Dairy Council in Chicago in 1915 was on hand to receive special honors. He is O. E. REED, director of livestock research, USDA, who received an honorary life membership certificate.

"Patron Saint" of the dairy industry is the name given McCollum, whose discovery of vitamin A in 1913 began continuing nutrition research which has revealed the essential importance of milk in the diet. Later, his leadership helped bring about the Dairy Council industry-approved program of research and education in dairy foods.

Dr. Hunt was cited as an outstanding leader in education who has cooperated with NDC in research and consumer education in nutrition. Dr. Stiebeling was selected among government leaders; Dr. Parran among professional leaders; and Mrs. Leonard among consumer leaders. Secretary Benson's citation was for his advocacy of research and education as a means of strengthening American agriculture.

Major Dairy Conventions to Return to Atlantic City in Fall of 1956

The 20th Dairy Industries Exposition in concurrence with conventions of major dairy processor organizations will be held in the autumn of 1956 in Atlantic City. The most recent Dairy Industries Exposition was held last fall in Atlantic City, and there has been some industry speculation as to whether in 1956 it might revert to a mid-western site, Chicago having been its location in 1952. The general understanding in the industry, however, is that the hotel interests in Chicago had failed to clear

accommodations for a week into which the cycle of operations in the dairy processing field would permit the conventions and the Show to fit.

The 1955 annual conventions of Milk Industry Foundation and Intern. Assoc. of Ice Cream Mnfrs. will be held in October in St. Louis. The annual convention of the National Assoc. of Retail Ice Cream Mnfrs. will be held in November, 1955, in Milwaukee.

News from Iowa State

C. F. FOREMAN was appointed assistant professor on the dairy husbandry staff at Iowa State College on January 1. After receiving the B.S. and M.S. degrees at Kansas State College, he served at that station as extension dairyman. Later he obtained his Ph.D. degree from the Univ. of Missouri, then joined the dairy husbandry staff at Univ. of Minnesota. At Iowa State College Dr. Foreman will do teaching and research.

H. J. LARSEN (Ph.D. Iowa State College, 1953), who was instructor and associate in dairy husbandry at Iowa State College, has joined the staff in dairy husbandry at the Univ. of Wisconsin.

H. H. VOELKER, formerly graduate assistant at Iowa State College, has been appointed assistant professor of dairy husbandry at South Dakota State College.

C. J. BLACKBURN, who received the fellowship awarded to Mississippi State College by the Dairy Industry Supply Assoc. in 1951, has begun his graduate studies in dairy bacteriology at Iowa State College. He recently was discharged from the army after seeing service in Japan and Korea.

Borden Expands Chemical Division

The Borden Co. has added to its chemical division through the acquisition of American Monomer Corp. and Monomer-Polymer, Inc. Both companies, which are located in Massachusetts, specialize in research and the production of thermoplastic and thermosetting material.

Dairy Products Improvement Institute Elects Wentworth President

The Dairy Products Improvement Institute, Inc., held its 8th Annual Meeting in the East Ballroom of the Hotel Commodore January 27. W. A. WENTWORTH, The Borden Co., New York, was reelected president. A. C. FISHER, General Ice Cream Corp., Schenectady, N. Y., was elected vice-president; R. C. HIBBEN, Intern. Assoc. of Ice Cream Mnfrs., was reelected treasurer; and A. C. DAHLBERG, Cornell Univ., was reelected secretary.

Dry Milk Institute to Hold Meeting

Members of the American Dry Milk Institute will hold their annual meeting April 19-20 at the Edgewater Beach Hotel, Chicago.

News from Louisiana State University

The L.S.U. Dairy Improvement Center, headquarters of the Louisiana Artificial Breeding Coop., received an excellent rating recently after inspection by a representative of the Purebred Dairy Cattle Assoc. Maintaining the highest standards in artificial insemination in purebred herds through the adoption of uniform rules and regulations is one of the major projects of the Association. The Center was congratulated on the quality of the bulls, the outstanding program for proving young bulls, the excellent condition of the laboratory building and grounds, and the handling of records. Eleven bulls each of the Holstein, Jersey, and Guernsey breeds are at present serving 61 of the 64 parishes of the state.

L. L. RUSOFF, professor of dairy and nutritional chemistry, has been elected chairman of the Baton Rouge Section of the American Chemical Society.

The dairy judging team of L.S.U. outscored five competing schools, with a total of 1,847 points at the 1955 Southwestern Exposition and Fat Stock Show and was named champion of the dairy judging contest for senior agricultural colleges.

High man was L.S.U.'s H. H. LACOBEE, JR., with 634 points, 10 ahead of his teammate, CHARLES CLARK. The team also took top honors in judging of Jerseys and Guernseys. The team was coached by J. B. FRYE, JR., head L.S.U. Dairy Department.

Anderson Appointed to USDA Dairy Division

DON S. ANDERSON has been appointed director of the Livestock and Dairy Division of USDA's Commodity Stabilization Service, effective the first of this year. He has been serving as acting director, prior to which he had been deputy director since May, 1946, of the Dairy Branch, Production and Marketing Administration.

Anderson taught at the Univ. of Minnesota and at the Univ. of Wisconsin. He is a veteran of World War I, having served as an infantry lieutenant. In his work with the federal government he has been with the AAA, OPA, and WFA, all of which were concerned with some phase of the dairy industry, and he has actively participated in the huge dairy price support purchase programs.

New Departments to Be Added

As an added service to its readers the Journal of Dairy Science is adding two new departments. "The Bulletin Board" will offer opportunities for members and subscribers to place classified advertisements for the sale of property and equipment, for positions open, and positions wanted. Rates will be supplied upon request.

A professional directory will be made available to dairy and food consultants desiring advertising space in the Journal. The standard space will be 1/12 page. The rate will depend upon the number of insertions per year.

An index to advertisers will be started in an early issue. Readers are urged to give special consideration to advertisers whose financial support makes it possible to sell the Journal at its present low price.

British Scientists Study Cheesemaking Here

T. R. ASHTON, technical advisor of the Express Dairy, Ltd., London, and WALTER NELL, chairman and governing director, recently spent some time in the States observing cottage cheese manufacturing operations. At present cottage cheese is not made in England in large quantities, but it is the plan of the Express Dairy to introduce the product to its London customers in the near future.

University of Tennessee Survey of Graduates

The Dairy Department of the Univ. of Tennessee has canvassed its alumni to determine their present occupations. The summary of this report follows:

	Per cent
Farming (owners, breeders, and herdsmen)	15
Extension (specialists, county agents, and assistant county agents)	15
Teaching and research (colleges, schools, and experiment stations)	13
Dairy manufacturing (owners, operators, and managers)	10
Fieldmen and sanitarians (processing plants and health officials)	13
Administrators (managers and executive secretaries)	12
Salesmen (equipment, supplies, feed, etc.)	5
Miscellaneous	10
Armed Services	7

Milk Marketing Order Appointments

The USDA has announced the appointment of W. W. HURWITZ as administrator of the Akron, Ohio, Milk Marketing Order. He formerly was chief auditor for the Cincinnati Milk Marketing Administration.

C. C. TAYLOR has been named administrator for the Austin-Waco, Texas Milk Marketing Order. He formerly was acting administrator of the Knoxville order.

Federal Milk Orders have been recommended by the USDA for Shreveport, La.; Eastern South Dakota; Amarillo, Texas; Wheeling, W. Va.; Steubenville and East Liverpool, Ohio; and upstate Michigan.

News from Ohio State

A meeting of university staff members and industry representatives was held in conjunction with the Ohio Dairy Products Convention in Cincinnati on Jan. 24 to discuss a state-wide program to interest more young men in careers in the dairy industry. Plans were laid for the 2nd Annual Dairy Technology Career Day on April 16. Definite plans were proposed to combine the efforts of the Ohio Dairy Products Assoc. and the Dairy Boosters Assoc. in the program.

Three scholarship awards have been made to undergraduate students: JOHN R. WISH, a junior, received the Ohio Booster Award for outstanding campus leadership displayed during his first 3 years at the University; HAROLD R. OBERLIN, a sophomore, received an R. B. Stoltz Award for his outstanding scholarship and his exceptional leadership in student activities; RICHARD THIELMEYER, a sophomore, received an R. B. Stoltz Award for having the most outstanding scholarship record of any student majoring in dairy technology.

The Department has received a research grant from Swift and Company to further the fundamental research on milk proteins.

Recent Deaths

J. C. HENING, associate professor in the Department of Food Science and Technology at the New York State Agricultural Experiment Station at Geneva, died February 17 after a long illness. He was born at Stillwater, Minn., in 1891. He obtained a bachelor's degree at Minnesota in 1917 and a master's degree at Wisconsin in 1923. For a number of years he was associated with the dairy research program at Geneva. From 1943 to 1946 he served as food technologist of the research and development laboratory of the Quartermaster Corps of the U. S. Army in Chicago. Since 1946 he had been working in the field of food technology at Geneva.

HARRY D. DRAIN, head of the dairy testing laboratory for the Ohio Department of Agriculture, Columbus, died suddenly February 28 at the age of 68. Mr. Drain graduated from Ohio State in 1913 and obtained his M.S. degree in 1924. He was on the staff at Ohio until 1929, when he joined the Borden Company as production manager of the Akron, Ohio, plant. He retired from the Borden Company in 1951 to accept the position with the Ohio Department of Agriculture.

Hollender Joins Food and Container Institute Staff

H. A. HOLLENDER, former associate professor in the Dairy Department at Purdue Univ., has been appointed Chief of the Dairy, Oil and Fat Products Division, Food Laboratories, Quartermaster Food and Container Institute, Chicago. This position was formerly held by J. M. MCINTIRE, who has joined the research staff of the Carnation Milk Co.

Hollender is a graduate of Michigan State. He obtained his M.S. degree in Dairy Technology at the Univ. of Illinois and his Ph.D. at the Univ. of Wisconsin.

James Smith Joins Creamery Package

JAMES SMITH recently resigned his position as plant manager of the Ohio State University creamery to become sales engineer for the Creamery Package Mfg. Co. A graduate of Ohio State, Smith spent several years as zone supervisor for Seal Test.

Completed Theses

M.S. Degree:

G. D. O'DELL—The effect of glycerol equilibration time on the freezing of bovine spermatozoa in egg yolk–sodium citrate and skimmilk semen diluters. Clemson College, South Carolina.

T. R. SPANN—The effect of rapid growth with fattening upon lactation in cattle and rats. Univ. of Tennessee.

J. H. WILLIAMSON—A comparison of two methods of milk feeding, with and without rumen inoculation, upon the growth and feeding efficiency of dairy calves. Univ. of Tennessee.

T. W. HOLZINGER—Secondary growth in a bacteriophage-lysed strain of *Streptococcus lactis*. Iowa State College.

Ph.D. Degree:

T. G. MARTIN—Factors affecting growth rate of dairy calves. Iowa State College.

THORVALD KRISTOFFERSEN—Degradation of amino acids by *Lactobacillus casei* in relation to flavor development in Cheddar cheese. Iowa State College.

Opportunities in Dairy Manufacturing

A Guest Editorial

There are more than 60,000 processing plants in the United States that purchase milk and distribute it to the consumer in the form of fluid milk, evaporated milk, cheese, butter, condensed milk, ice cream and similar foods. They process more than 50,000,000,000 qt. of milk, or their equivalent, each year. That amount of milk would fill a river 3,000 miles long, 40 ft. wide, and 3 ft. deep. More than 250,000 people are employed in the dairy processing plants. More than 190,000 trucks and at least 15,000,000,000 bottles are used annually by the dairy industry.

J. J. Sheuring

Some of the job opportunities for young men and women seeking a career in the dairy manufacturing phase of the industry include the following:

Procurement. This department is responsible for securing sufficient milk to meet the consumer needs. Personnel should be trained in both dairy production and dairy manufacturing. Many of the dairy companies employ trained men specifically for this type of work and usually give them the designation "fieldmen." They are extremely important in establishing good relations between the dairy processors and the dairy producers.

Production. This department receives and processes the milk and manufactures the various milk products. Workers in this field include plant superintendents, department foremen, and specialized workmen, such as operators of ice cream freezers, pasteurizers, and bottle fillers. These jobs require young men who are technically trained and who can properly supervise and instruct subordinates. They also must have the ability to organize for maximum production at a low unit cost.

Laboratory. The dairy industry is very closely supervised by regulatory officials to insure that the consumer secures a safe, clean, and nutritious product. Most of the large dairy plants and many of the smaller ones maintain laboratories for accurate control of the quality of their products. This creates jobs for young men and women who are especially interested in bacteriology, chemistry, and the general field of quality control. Many companies also conduct rather extensive programs of research which come within the scope of the laboratory operation.

Inspection. State and city health agencies employ many persons to inspect dairy farms and plants from a health and sanitation standpoint. This type of work requires personnel who are trained in bacteriology and the fundamentals of dairy production and processing. Some veterinarians and engineers are employed for this type of work, especially in the military services. There is a need for young people who have a dairy background to continue graduate work with the purpose of securing an advanced degree in public health.

Engineering. The planning of dairy plant construction and maintenance of equipment is a major phase of the dairy industry. The opportunities for young men with proper training in this important part of the dairy industry are almost unlimited. Many dairy plants maintain shops to repair and develop equipment, including electrical, refrigeration, automotive, and specialized types of machinery. A fundamental training in mechanical engineering and dairy manufacturing is important for those who are interested in this type of work.

Accounting and office management. The importance of adequate records in any business cannot be overstressed. The dairy industry is large and diversified and must keep a great variety of records. Practically every dairy plant must employ a bookkeeper, and the larger plants maintain a well trained staff of accountants, comptrollers, bookkeepers, record clerks, and credit managers. A basic training in business administration, including business machine operations, and a fundamental training in dairying are important for the key personnel in this important phase of dairy plant operation.

Merchandising. The selling of the product to the consumer is the final goal of the dairy business. This phase of the industry offers wonderful opportunities for men and women who are trained in merchandising, public relations, and journalism. Millions of dollars are spent annually by the dairy industry to merchandise its products, and there is a demand for both men and women who are interested in selling. There are opportunities for women in dairy councils, on television programs, and as official hostesses. A basic training in home economics with a minor in dairying is desirable for women interested in this type of work.

Labor relations and personnel. This is another field that is becoming more and more important in the dairy industry, and many dairies employ men and women especially for this work. A basic training in human relations, supplemented by training in dairying and business administration, is desirable for this type of employment.

Management. This is the ultimate goal of most people in any industry. The dairy industry offers great opportunities for advancement into executive positions. For the young man or woman who is willing to be trained and to work diligently and faithfully for a dairy company, the rewards will be rich. To attain an executive rating with a large dairy company, an employee must be of outstanding character and have executive ability.

Research and teaching. For those who are interested in pursuing graduate study there are excellent opportunities in the fields of dairy research and teaching. Many commercial organizations maintain a regular staff of research personnel. Colleges and universities are always interested in securing well trained persons for their teaching and research staffs. This field should be considered by young people who are interested in completing the requirements for a doctor of philosophy degree.

There is an excellent future for properly trained young people in the processing, management, research, and sales phases of the dairy industry.

J. J. SHEURING
Department of Dairy Husbandry
University of Georgia

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Will No-Hold Pasteurization Come Next?

JOSEPH TOBIAS

Department of Food Technology, University of Illinois

In recent years a considerable amount of work has been done on equipment capable of heating milk rapidly to temperatures much higher than those required by present pasteurization standards. Although some phases of application of this new process have not gone much beyond the pilot plant stage, it seems desirable to review some of the findings and speculate as to future developments.

Heat Affects Most of the Constituents of Milk

Heat-induced changes in milk are dependent on both temperature and time of exposure. A well-established principle is that as the temperature is increased the time necessary to bring about a certain change is decreased. This holds true whether the change is microbiological or chemical. To fully appreciate the significance of the time-temperature relationship, it is necessary to know the changes which are likely to occur.

Changes due to heat may be manifested in a number of ways, such as:

Destruction of bacteria
Inactivation of such enzymes as phosphatase, lipase, peroxidase, and catalase
Reduction in the vitamin content, particularly vitamins C, B, and possibly A
Changes in the salt balance, especially involving calcium, phosphorus, and the citrates
Development of a brown color due to a protein and lactose interaction
Reduction or loss of cream line
Development of heated and cooked flavors
Denaturation of whey proteins
Reduction in curd tension
Activation of reducing sulfhydryl groups with a corresponding decrease in the oxidation-reduction potential
Lengthening of rennet coagulation time
Variation in gas content
Formation of organic acids
Changes in the water-binding capacity of casein
Hydrolysis of proteins, fats, and lactose
Changes in the structure and charge of colloidal particles

Some changes, such as the production of off-flavors, may not be detected immediately after heating but make their appearance during the storage life of the milk. There is much still to be learned about the effect of heat upon milk, and research no doubt will bring to light changes of which we are not aware at the present time.

The extent of heat treatment necessary to bring about these changes varies. Thus, it would be virtually impossible to sterilize milk at a temperature of 143° F., whereas such enzymes as lipase and phosphatase are readily inactivated. The rate at which changes take place varies with increasing temperatures. Reduction in cream line volume proceeds rapidly as the temperature is raised, but the development of cooked flavor and reduction in curd tension follows a much more gradual pattern.

Heat-induced changes are not brought about solely by an exposure to the top heating temperature, as there is a cumulative effect which includes heat-up time, holding time at top temperature, and cooling time. Slow heat-up and cooling times may be responsible for a good share of the changes resulting from a particular heat treatment. It becomes evident that much better control of a process may be achieved by reducing heat-up and cooling times to a bare minimum, depending upon exposure at the top temperature to do the desired job. Equipment has been introduced recently which is capable of heating milk to temperatures of 300° F. or higher in a few seconds, after which it is immediately cooled in an equally short period of time. The high temperatures attainable make it possible to eliminate holding time entirely from such processes as sterilization of milk or pasteurization of ice cream mix. Figures 1 and 2 give illustrations of no-hold heating equipment which may be used to process milk at both pasteurization and sterilization temperatures. The Vacreator, shown in Figure 3, does not employ sterilization temperatures but is essentially a no-hold system of pasteurization.

Increasing the Storage Life of Milk

The fundamental problem of increasing the storage time of milk can be solved only by the elimination of bacterial life without loss of good flavor. Momentary exposure to temperatures above the boiling point shows promise of furnishing this desirable objective. The ulti-

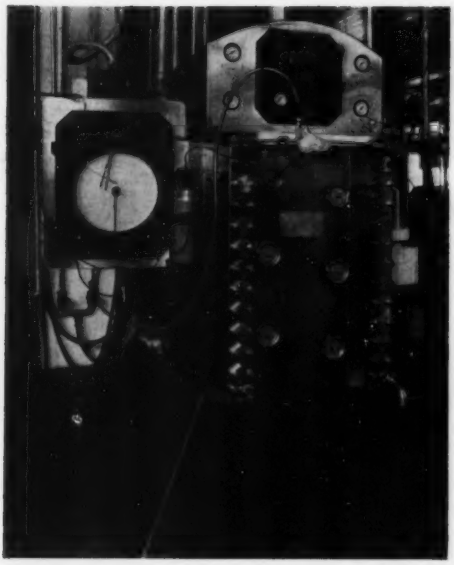


FIG. 1. The Mallorizer is an example of a tubular type heater used in high temperature processing. Milk is pumped through a small diameter tube at high velocity creating a turbulent flow of liquid. Processing temperatures employed range from below the boiling point to over 300° F. The unit may be operated with or without a holding tube. The model illustrated above will process 200 gal. of product per hour with a 6-second heat-up time and a 6-second cooling time. It is capable of raising the temperature of the product by approximately 180° F.

mate goal, of course, is to have milk that will keep when stored at room temperature. This requires a complete absence of viable organisms and the prevention of any physical and chemical changes which may result in off-flavor production.

At the present time sterile milk is made experimentally by momentary heating to a temperature in the vicinity of 300° F. followed by aseptic canning or bottling. The milk may be homogenized either prior to or after sterilization, the latter requiring an aseptic homogenization process. No data have been made public to date to establish the merits of one procedure over the other. Homogenization before sterilization would be preferred for its greater simplicity, providing the alternate method did not result in a better emulsion stability.

Milk processed in this manner is not discolored or thickened and has generally the appearance of fresh homogenized market milk. Its flavor initially is somewhat comparable to that of regular pasteurized milk, the principal off-flavor being cooked and of the order of magnitude found in over-pasteurized milk. The intensity of the cooked flavor has a tendency to diminish upon storage or exposure to sunlight. When stored at room temperature, sterile milk is subject to flavor deterioration. Within a few weeks a distinct old or stale flavor develops, which may or may not be superseded by a true oxidized flavor. The flavor evolution appears to be oxidative in nature, which suggests some obvious corrective measures, such as deaeration during processing and bottling, the use of antioxidants, and exclusion of light and catalytic metals. The storage life of the milk is much longer at 40° F.

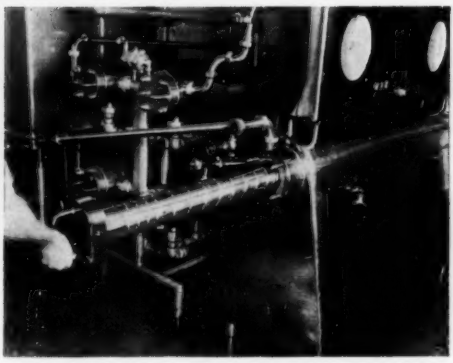


FIG. 2. The Roswell Heater represents another type of equipment suitable for high temperature processing. A film of milk ⅛ in. in thickness passes in a spiral fashion between two heating surfaces, resulting in rapid heat-up and turbulent flow. The unit may be operated within a wide range of temperatures above and below the boiling point, with or without a holding tube. The model illustrated has variable capacity of 5,000 to 7,000 lb. per hour and is capable of a heat transfer of approximately 700,000 B.t.u. per hour. Heat-up time is 1.8 seconds when the heater is operated at 6,000 lb. of product per hour.

Of considerable interest are the results obtained by heating milk in the region of 240° to 250° F. with a momentary exposure. These temperatures do not guarantee sterility, but good quality milk subjected to such a treatment may show a bacterial count of a few to less than one organism per milliliter. The milk would be bottled in the same manner as pasteurized milk and, if post-heating contamination is eliminated by scrupulous sanitation practices, the product should have excellent keeping quality at 40°-50° F. The result should be a storage life of 3- to 5-week duration.

Under the above conditions there should be no difficulty with positive coli or phosphatase tests. Available data indicate that rapid heating to 176° F. with a holding time of 0.5 second will result in satisfactory inactivation of phosphatase.

Processing of Cream and Skimmilk

Of the many perishable types of dairy products, various types of cream especially should have good keeping quality. In spite of its higher viscosity, cream may be satisfactorily processed at high temperatures, as it does not seem to burn onto the heating surface as readily as do products containing a lower fat content. The sterilized cream may be canned aseptically or may be marketed in the same manner as suggested for the nearly sterile milk.

Skimmilk is the most difficult product to process in rapid heat exchangers. It has a tendency to form a build-up on the heating surfaces with a resulting rise in pressure which eventually necessitates a shutdown. Possible explanations for this phenomenon are lack of protective lubrication of the heating surface, precipitation of insoluble salts at high temperatures, presence of air and other gases, high acidity, improper salt balance, or a combination of these factors. Various attempts are made to solve this problem, and perhaps some combination of treatments, such as de-aeration and step-wise heating, will lead to a successful solution.

Ice Cream Mix Treatment

It has been shown that ice cream mix may be successfully processed by no-hold heating methods. Temperatures higher than necessary to give bacterial destruction comparable to conventional pasteurization methods have been found to give best results from the standpoint of flavor and body and texture of the frozen ice cream. In one study employing the Mallory small tube heat exchanger with a 6-second heat-up time, a temperature of 185° F. gave satisfactory bacterial destruction, but the ice cream made from the mix lacked the desirable body and texture found in that made from mix processed by the long-hold method. An increase in heating temperature to 240° F. resulted in marked improvement in body and texture without deleterious effect on the flavor. Higher processing temperatures in this heater were detrimental to flavor, and the ice cream possessed an excessively dry body. Ice cream makers have long been aware of the beneficial effects of superheated concentrated milk in their product, and it is conceivable that in very high temperature processing the entire mix is in effect superheated. If such is the case, one may expect additional benefits from stability against heat shock and possibly shrinkage.

Stabilizers and other dry ingredients must be incorporated in the cold mix unless provisions are made for metering previously prepared solutions of these substances into the line or unless a vat is used for preheating. It may be found that the high-heat treatment affects the performance of some stabilizers in respect to viscosity, whipping quality of the mix, and body of the ice cream.

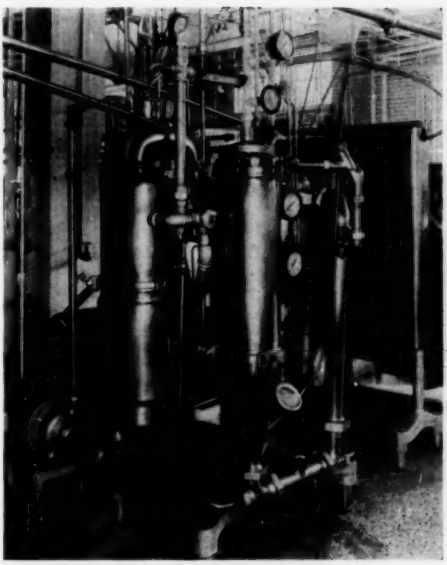


FIG. 3. The Vacreator is used at temperatures below the boiling point. Heat transfer is accomplished by mixing milk with steam under reduced pressure. The condensed steam is volatilized by subsequent vacuum treatment. No holding tube is employed in this system. The model illustrated above has a rated maximum capacity of 3,000 lb. per hour.

Application of High Temperature Heating to the Manufacture of Other Dairy Products

Studies have been made on the effect of very high temperature preheating of milk intended for sweetened condensed milk to be used in the manufacture of caramels. Heating to 300° F. in 6 seconds without holding proved beneficial, as judged from the body and flavor of the caramels.

Skimmilk intended for cottage cheese manufacture heated instantaneously in the region of 190° to 200° F. has produced a very good acid curd cottage cheese. Chocolate milk made by high temperature processing has been found satisfactory. It is possible to make a sterile product by this method, though undesirable flavor changes may occur during storage.

There are other possible applications of very high temperature heating which undoubtedly will be explored in the future. The method may fit into the manufacture of dry milk.

Public Health Considerations

New pasteurization standards will have to be formulated and approved by health regulatory agencies before no-hold processing can replace present methods. Accurate temperature control

will be an important requirement; only the holding tube will be eliminated. The contribution of heat-up time to the destruction of bacteria will become an important consideration, and it probably will have to be evaluated in terms of its equivalency to the processing temperature. It will be necessary to know the lowest temperature to which the product should be heated with no more than 0.5-second holding time to give results comparable to those obtained by accepted pasteurization methods. The flow diversion valve control could then be set at this temperature to comply with minimum pasteurization requirements, even though the product may be processed at considerably higher temperatures at the option of the operator.

TABLE 1

Pasteurization temperatures required to achieve expressed objectives in the processing of milk and milk products using a ½-second holding period

Objective	Milk	Cream	Ice Cream Mix
	(° F.)	(° F.)	(° F.)
Negative phosphatase [1]	176	177	180
Destruction of *Myc. tuberculosis* [2]	175	178	180
Destruction of *E. coli* [3]	175	—	—
Inactivation of lipase [4]	175	—	—
Cream volume reduced [2]	174	—	—
Cream line destroyed [5]	194	—	—
5% whey protein denaturation [4]	190	—	—
50% whey protein denaturation [4]	244	—	—
75% whey protein denaturation [4]	325	—	—
Soft curd [4]	290	—	—
Zero curd tension [4]	332	—	—
Minimum pasteurization temperature	180	185	195
Possible 1 mo. stor. life at 40° F.	240	250	—
Possible adverse flavor	>240	—	>250
Superheating	—	—	255
Sterilization temperature	295	300[a]	300[a]

[a] approximate

[1] Sanders and Sager, *J. Dairy Sci.*, **31**: 845. 1948.

[2] North and Park, *Am. J. Hyg.*, **7**: 147. 1927. Oldenbush, Frobisher and Shrader, *Am. J. Pub. Health*, **20**: 615. 1930.

[3] Holland and Dahlberg, *Tech. Bull.* 254, N. Y. St. Agr. Expt. Sta., Geneva. 1940.

[4] Hetrick, Ph.D. thesis, University of Illinois. 1947.

[5] Harland, Coulter, and Jenness, *J. Dairy Sci.*, **35**: 363. 1952.

Pasteurization temperatures required to achieve certain objectives in the processing of milk and milk products are given in Table 1. Most of the values reported were obtained by linear extrapolation of data found in the literature, using a 0.5-second holding time and assuming a heat-up time of the order of 1 second. Under these conditions, the minimum pasteurization temperature for milk should be 180° F. to insure a negative phosphatase and coliform test and to maintain the safety margin afforded by present pasteurization methods. Other expected changes that would be brought about by this heat treatment include the destruction of *Mycobacterium tuberculosis*, inactivation of lipase, some reduction in cream volume (complete destruction of the cream line would occur at a temperature of 194° F.), and less than 5% denaturation of whey proteins. Sterilization of milk would require a processing temperature of 295° F., which, in addition to sterility, will produce a soft curd milk with between 50 and 75% of the whey proteins denatured.

The minimum pasteurization temperature of ice cream mix should be 195° F. in order to provide the same margin of safety afforded by the U.S.P.H. standards of 155° F. for 30 minutes and 175° F. for 25 seconds. At a temperature in the neighborhood of 255° F., a superheating effect will be observed, as manifested by an improved body and texture of the ice cream.

Higher temperatures are required for cream than for milk, apparently because of the protective action of the cream solids. The minimum pasteurization temperature for cream is 185° F. when using a 1-second heat-up time and a 0.5-second holding time. To sterilize cream, a temperature of about 300° F. is necessary.

The Heater of Tomorrow

Most of the heaters being studied allow milk and other fluids to be processed at temperatures as low as 160° F. and as high as 300° F. Somewhere within that range lies the optimum temperature necessary to accomplish a prescribed task, be it to sterilize milk or to pasteurize ice cream mix. Within that range there is also a temperature that will bring about an improved keeping quality of perishable products and an improved body and texture of manufactured products. As more is learned about this process, the possibility arises that some day pasteurization may mean the destruction of all bacteria in milk rather than the killing of all pathogenic organisms and most of the nonsporeformers. Fast heating to high temperatures followed by fast cooling undoubtedly will be the method used in dairy plants of tomorrow.

Dairy Herd Health

Simple Rules for Keeping a Herd Healthy

RAY D. HATCH
Department of Veterinary Clinical Medicine, University of Illinois

One of the most elementary rules for maintaining a disease-free herd is to maintain a closed herd. That is, buy no animals or buy as few as possible. If a herd must be enlarged and the increase can be anticipated, purchase immature, unbred heifers. Open and immature heifers are less likely to harbor organisms of disease that can be disseminated to the milking herd. Neither the reproductive tract nor the mammary gland is sufficiently developed in the heifer calf to harbor or permit the growth of organisms (bacteria) most likely to cause epidemic diseases in a dairy herd. The two groups of disease conditions most important to the dairyman are mastitis and infertility, which is just a broad, all-inclusive term for all breeding disorders.

Buy Only Disease-Free Cattle

Obviously, if only disease-free animals are to be purchased, the purchaser must know the health status of the herds from which replacements originate. If the seller has a disease-free herd he will generally be quite proud of that fact and will have no misgivings about discussing it. Every owner who has recently had his herd tested for brucellosis and found all animals free of the infection will gladly show a prospective purchaser the test charts and results. However, a dairyman with 10% infection in the herd will be reluctant to display the test results for all to see.

No Visitors—a Good Policy

A policy of no visitors might also aid in reducing the spread of some diseases. Although there are no anti-social introverts among dairymen and strict observance of such a policy is impossible anyhow, at least there are some circumstances that will justify curtailment of such traffic. To point an example, many outbreaks of Newcastle disease in poultry occur 3 or 4 days after the delivery of a new batch of feed. The feed truck had delivered feed to a flock owner whose birds were infected and carried the infection mechanically. Although there is no disease of dairy animals as readily transmissible as Newcastle disease of poultry, this is an example of one way in which disease can be transported. Keeping visitors and foreign equipment to a minimum will prevent some disease spread.

Obviously it is necessary to purchase some items and on certain years much of the feed for the herd must be bought. Many disease organisms can be transferred by feed; hay from an anthrax-infested area may be infective. This does not imply that all feeds are capable of causing a severe disease but it is an argument in favor of the home production of as much feed as possible.

Test for Disease Regularly

Regular testing programs also should be followed. The present federal and state testing programs are aimed at two of the major scourges of dairy animals—tuberculosis and brucellosis—but they must be applied regularly to be of most value. The herd owner should see to it that these tests are carried out. Records of tests and dates may be lost in the press of other duties, and herds may go for years without a retest. Part of the responsibility for the application of the test is up to the owner. Although private tests are at the expense of the owner, they are generally well worth the cost. Many outbreaks of brucellosis have been controlled or prevented by an annual (or more frequent) herd test. Without cooperation from the producer no disease eradication program can be a success. The blood test for brucellosis does not detect all of the causes of breeding trouble but it does eliminate an important cause; fortunately most breeding diseases can be controlled by the same simple rules that are used to control brucellosis.

Isolate Animals at Calving Time

All animals, regardless of disease status, should be isolated at calving. If there are any breeding diseases in a herd they will be transmitted at this time. Most of the organisms causing genital infections are present on the fetal membranes if they occur in the genital tract. The membranes and fluids should not contact other animals. No animal should be put back in the herd if there is a vaginal discharge, as these secretions may be just as infective as the heavily contaminated membranes.

Breed Only Healthy Cows

Animals showing abnormal genital discharges or secretions at heat should not be bred. Such animals will not breed normally and often will abort after a short period of pregnancy. A great many animals with a known history of uterine infection conceive only to give birth to dead or weak, nonviable calves. Only the healthy cows should be permitted to produce offspring. Not all abnormal discharges are infectious, but because many are, the risk is too great for the average producer.

Avoid Injury to Animals

Of the many causes of injury to animals, the accumulation of junk, broken equipment, and rolls of wire, sharp sticks, or nails around the barn and lot is one too often encountered. Nothing is more easily remedied. Many dairymen pay out large sums annually to have wounds sutured and post-injury infections treated; the removal of the offending objects would be far more economical. The cost of repair and the loss of production in one cow that injures the udder on an old tractor wheel or cuts a teat on a roll of barbed wire are generally far greater than the cost of removing the material. The loss in production alone will pay a week's wages for a man employed to clean up the area.

Nails and wire create a hazard in another way also. Cattle consume much of such material if it becomes lodged in the grass the animals eat. Much of this extraneous material causes no trouble or only a temporary indisposition, but some will have serious consequences. The better producing cows are most often affected because they are the most voracious feeders.

FIG. 1. Poor sanitary conditions in this barnyard resulted in the development of leptospirosis in these calves.

They are also the animals producing the better heifer replacements for the herd—but a dead cow produces no calves.

Another area that should receive attention is the mud hole or badly washed area. Drainage, downspouting, and watering tank construction are engineering functions which are important to herd health. Washes around the approaches to a barn make for high door sills or sharp breaks in the approach and thus provide a possibility of udder injury. Even minor chronic injuries may lead to serious changes in the udder. Foot rot is more prevalent on farms or around areas that are continually wet; moist, soft skin between the digits is more susceptible to injury, and if the organism is present the likelihood of disease is increased.

One particularly difficult area for drainage is around the water tank. If the animals have to stand in mud (during the winter) they consume less water, resulting, of course, in a decreased milk flow. Water is one of the least

FIG. 2. An assortment of hardware that these two veterinarians have taken from the stomachs of mature cows.

expensive items supplied to animals and also one of the most important. Many dairy cows can go off feed and down in production simply because a drinking fountain does not work properly. A valve may stick, hay may accumulate in the bowl, or possibly an electrical short or ground may occur. Be sure that all fountains work properly and that all animals have an opportunity to drink at the tank.

The type of material used as a fill around the water tank also can be a hazard. Coarse, broken building material, bricks, or concrete blocks can be used if the coarse material is well covered with sand or gravel. Sharp, rough particles are likely to cause foot injury; also, if the footing is not comfortable, the animals will not consume adequate amounts of water.

Care of Udder Important

Any discussion of dairy herd health must include the care and health of the udder. The mammary gland of the domestic dairy cow is an artificial organ, produced by years of selective breeding. In this selection breeders have neglected to consider that cows have lost some of the normal resistance to udder diseases that is present in the breeds of animals not noted for milk production. The better dairy cows have large udders; any injury, regardless of severity, can have serious consequences.

One of the more important areas of the udder, from the standpoint of disease, is the teat orifice. Most udder infections develop from bacteria gaining entrance into the milk ducts through this opening. Large openings, which permit easy milking, also permit organisms to get into the system more easily. As a result, there is a correlation between ease of

milking and incidence of mastitis. Of course there are other factors: some animals may have a systemic resistance to certain bacteria, or some bacteria may have a higher degree of invasiveness.

Anything that can be done to prevent bacteria from contacting or accumulating on or around the teat opening will aid in preventing mastitis. After milking is completed, the teat orifice may be partially everted, or turned wrong side out, but in a few minutes it returns to a normal position. If mastitis-causing bacteria happen to contact this everted opening they will eventually enter the teat canal and advance from there to the secretory tissue of the udder. A drop of milk at the teat opening may be sufficient to attract flies, and as they move from one cow to another they could spread the infection.

Mud, manure, and other foreign matter also may accumulate around the end of the teat. If any of this material is drawn or forced into the tiny teat canal, infection can result. Even the treatment of mastitis by inserting tubes through the teat opening may force infectious material into the teat cistern. The skin of an animal is rarely free of bacteria, and flakes of epithelium that are loosened and forced into the teat also may carry organisms.

If mastitis does occur and it is necessary to treat the infection, it is impossible to be too careful. The end of the teat and the teat opening should be carefully cleaned and sterilized before administering any drug. Even the choice of antiseptic or sterilizing agent may be a hazard. Some antiseptics are caustic or irritating and cause chafing or cracking of the teat. A longitudinal fissure or crack in the epithelium of the teat opening may be injury enough to permit an infection to develop. The healthiest udders are those kept clean and tampered with only to withdraw milk.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

171. The effects of some avian neurotropic viruses on young dairy calves. C. F. HELMBOLDT, R. E. LUGINBUHL, A. H. HAMMAR, S. F. SATRIANO, and E. L. JUNGHERR, Storrs Agr. Expt. Sta., Storrs, Conn. Am. J. Vet Research, **16**, 58: 57. 1955.

Twenty-five dairy calves were inoculated intrathecally and intracerebrally with viruses of Newcastle disease, avian encephalomyelitis, and equine encephalomyelitis. Newcastle disease intrathecally caused some fever and anorexia, but recovery was nearly complete in 2 days. ND virus could not be recovered from any of the inoculated calves. ND virus given intracerebrally caused serious nervous disorders, and 5 of 8 treated calves died. The virus could not be found at autopsy; so it apparently could not reproduce in calf brain. Avian encephalomyelitis caused no changes in the treated calves and could not be recovered from their brains; hence it is of no importance to bovines. Eastern equine encephalomyelitis virus was established in calves and could be recovered after a typical attack of the disease. It was concluded that the latter virus could be dangerous to the bovine population. E. W. Swanson

172. Analysis of milk in deficiency diseases and intoxications of cattle. W. T. BINNERTS and E. BROUWER. Nature, **174**, 4438: 974. 1954.

The authors recommend analyses for vitamins, trace elements such as Cu, I, Mn, Zn, Mo, As, Pb, and for insecticides and other toxic substances in excess in deficiency diseases and intoxications of cattle. Analyses are best made on mixed milk from creameries. Information thusly obtained frequently gives more reliable data than are obtainable from analyses of thyroid glands, urine, etc. R. Whitaker

BOOK REVIEWS

173. Dairy Cattle Feeding and Management. H. O. HENDERSON and P. M. REEVES. 4th edition. John Wiley and Sons, Inc. New York. 614 pp. 57 illustrations. $6.50.

This edition, 16 years after the third, carries a change in authorship to correspond to those active in the revisions. Thus, the present junior author appears for the first time on this edition, which is based on the original which appeared in 1917 by C. W. Larson and F. S. Putney. The authors have maintained the previous aim of providing students, dairymen, and others useful material in the field of dairy production.

The extensive revision has brought all parts to the authors' appraisal of modern dairying. To accomplish this task the number of pages has been increased by 57. The number of chapters remains constant at 35 with at least six distinctly new chapter headings and a corresponding number of deletions and rearrangements.

New concepts on herd management are added under such titles as milking the dairy herd, artificial breeding of dairy cattle, maintaining breeding efficiency, programs of the breed registry associations, the purebred dairy cattle business and marketing dairy cattle.

References for further study are included at the end of each chapter. Laboratory exercises with particular reference to each of the chapters are appended. Also included as appendices are tables of nutrient composition of common feeds (Morrison), recommended daily allowances for dairy cattle from two sources including N.R.C., various tables of weights and measurements of feeds, suggested cost figures for dairy farm operations, and an up-to-date gestation table. The index is of subjects only, as the footnote references are largely by source rather than by author.

Some of the technical errors of the third edition have been corrected in the present revision. The experienced user of this text may realize that the new errors that ignominiously appear for the first time might bother the authors more than himself.

In the opinion of this reviewer the microbiology of ruminant nutrition deserves more emphasis than is accorded the recent developments in this area. Some readers might prefer the more frequent use of quotation marks to portray adequate perspective. In general this text is easy to read and students with some background in dairying should easily grasp the salient features. It will serve a useful purpose for students taking their first course in dairy cattle management before having had chemistry and biology. L. O. Gilmore

174. Grundzüge der Milchwirtschaft und der Molkereiwesens. (Fundamentals of Dairying and Dairy Manufacturing) DR. GEORG ROEDER. Verlag Paul Parey, Spitalerstrasse 12, Hamburg 1, Germany. 830 pp. DM 96.80.

This book was written for the teacher and student, as well as for the consultant, research worker, and those concerned with the practical side of dairy manufacturing. It covers all important phases of dairying in a manner similar to that of the famous old text of Fleischmann and Weigmann. Quotations from 1,337 references indicate the thoroughness with which the author undertook his job.

The subject matter is treated along the following lines: The history of dairying; evolution of German laws and regulations pertaining to dairying; milk secretion; colloidal phenomena of milk; composition of milk; characteristics of the individual constituents of milk; physical constants of milk; changes in milk brought about by physical, chemical, and microbiological agents; bacteriology of milk and milk products; milking practices; processing of milk; butter manufacture; cheese manufacture; condensed milk and dried milk manufacture; utilization of by-products; water in the dairy plant; physical, chemical, and bacteriological testing procedures.

The author has made an effort to present the material as clearly and simply as possible and has used 559 illustrations to emphasize the important points. This is not to say that the book was written for those uninitiated in the sciences, as it is primarily a technical book.

As a reference book or as a text in schools where German is spoken, this book should be a valuable addition to the library. J. Tobias

175. The Lipids. Volume II. Biochemistry: Digestion, Absorption, Transport, and Storage. H. J. DEUEL, JR. 919 pages. Interscience Publishers Inc., New York, 1955. $25.00.

This book is the second of a three volume set which attempts to cover all the substances usually classified as lipids—fatty acids, glycerides, phosphatides, cerebrosides, waxes, sterols, fat soluble vitamins, hydrocarbons, pigments, etc. The first volume covered the chemical properties of these materials. The present volume is divided into six parts: introduction; the digestion and absorption of fats in the gastrointestinal tract; the digestibility of fats; the digestion, absorption, and digestibility of lipids other than fats; blood lipids; the occurrence of lipids in the animal as a whole; and lipid distribution in specific tissues and in their secretions. The third volume now in preparation will cover the biosynthesis, oxidation, metabolism, and nutritional value of lipids.

The author has attempted to be complete rather than selective. Classical as well as modern experiments usually are presented. The text is well supplied with references. Certain sections of the book are well integrated, but in some sections the text tends to become a compilation of data, frequently conflicting, and the significance of which is not always clear. Therefore, some sections are suitable for acquiring general background information and some are not.

There are good sections on the enzymes concerned with lipid digestion, bile, the various theories of fat absorption from the intestine, digestibility of fats, arteriosclerosis as related to dietary fat, body fat distribution as related to obesity, fatty livers and lipotropic factors, and the composition and biosynthesis of milk fat. There is also an extensive index.

This book should be of value to those engaged in the nutritional study of fats and fat soluble vitamins as a reference and key to the literature. E. G. Hammond

176. The Buttermaker's Manual. Volume I and II. F. H. MCDOWALL. New Zealand Univ. Press, Univ. House, Bowen Street, Wellington, C. I. 1590 pp. 1953.

The purpose of the author was to provide the buttermaker with "a study of all phases of the science and art of buttermaking and of butter factory practice, blended into a single coherent text to serve him as a source of reference on the problems with which he is confronted from day to day." The resulting manual certainly will go down as a milestone in the literature on buttermaking. The title is almost misleading because the book will be used as a reference source by a much wider circle of dairymen.

Chapters 1-17 cover the history and statistics of butter production; the composition of milk and butter; and the successive stages in the manufacture of butter, from the production of milk and cream on the farm to the packaging, shipment, and grading of the finished product. Especially noteworthy is a detailed and illustrated chapter dealing with the seven best-known continuous processes of buttermaking. Although this chapter was written three years before publication, the latest available information was included by means of footnotes and an addendum.

Chapters 18-24, well illustrated by tables and graphs, deal with the bacteriology of butter, the physical properties and structure of butter, and butter defects.

The second volume contains chapters 25-38, in which the following subjects are discussed: butterfat losses; butter yield; economic aspects of buttermaking; manufacture of ghee, dry butterfat, and related products; margarine; subsidiary operations in the creamery, e.g. manufacture of whey butter, cream, ice cream mix, and casein; heat, power, and water supplies; refrigeration; treatment and disposal of creamery wastes; management and operation of a creamery and factory testing and analyses. Volume II is supplemented by an appendix containing tables of useful data. Each volume has a complete index covering both volumes.

A detailed survey of the literature has been made and extensive lists of references are provided at the end of each chapter, grouped under subject headings.

The work is written clearly although the subject matter is voluminous and the discussions detailed. Because of the time required to compile so much information, some statistics are rather old. This refers in particular to tables on farm-made butter (Table 11), annual production (Table 16), and per capita consumption of butter (Table 20) in which figures for some or for all countries relate to pre-war years.

The size and price of the Buttermaker's Manual make it unsuitable for use as a textbook by undergraduate students. However, as a reference text it will be useful to college instructors, dairy inspectors, laboratory workers, or manufacturers of dairy equipment. To the creamery man it can well serve as his whole technical library. S. H. Lombard

177. Elements of Dairying. Laboratory manual. E. E. Bartley, F. E. Eldridge, E. L. Farmer, F. C. Fountaine, and G. B. Marion. Burgess Publishing Company, 426 E. Sixth St., Minneapolis 15, Minnesota. 127 pp. $2.25. 1954.

The manual contains 11 laboratory exercises covering the following areas: determination of fat and total solids in milk; observations of dairy manufacturing plant; quality evaluation of dairy products; feeding of dairy cows; feeding and management practices; dairy cattle housing; artificial breeding; and dairy cattle judging. It is not merely a laboratory manual, inasmuch as it contains a great deal of factual information which should be very helpful to the student. A general scheme is followed whereby the subject matter of the exercise is explained, a procedure for the experiment is given, and blank tables and charts are provided for the student to record his data. A number of pictures and charts also are included.

Some of the exercises were prepared to conform to the facilities available at Kansas State College, which would make certain modifications necessary if the manual were to be used at another institution. The layout of the dairy plant, for instance, would be different, but the changes could be accomplished easily without detracting from the usefulness of the book.

The manual should prove itself a valuable supplement in an elementary dairy husbandry course. J. Tobias

BUTTER

178. What about this business of butter weights. M. C. Escher, Mgr., Lakeside Butter Co., Chicago. Milk Products J., **46**, 1: 21. 1955.

Prepackaging of food in recent years has necessitated numerous changes in marketing methods and regulations. Weight and measure regulations established prior to present-day merchandising trends were based on hand-weighing and wrapping by the retailer at the time of purchase. However, prepackaging has resulted in the need for tolerances because of weight changes due to moisture loss over a period of time.

The author proposes that milk fat be made the basis of weight control in butter. One pound of butter (16 oz.) contains 12.8 oz. of milk fat (based on minimum of 80% fat). Since milk fat is the only valuable ingredient in butter this approach seems fundamentally sound. J. J. Janzen

CHEESE

179. Cheese packaging. G. N. Fisher (assignor to Kraft Foods Company). U. S. Patent 2,698,249. 1 claim. Dec. 28, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 4: 788. 1954.

Natural cheese in block form is enclosed in a plastic wrapper, then a paper board box is folded around it and pressure applied, to thoroughly seal the wrapping material around the cheese. R. Whitaker

180. Packaging. K. Bell and G. N. Fisher (assignors to Kraft Foods Company). U. S. Patent 2,699,285. 2 claims. Jan. 11, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 2: 233. 1955.

Cheese or other material in block form is wrapped tightly in a sheet of heat sealable film. A tear strip is imbedded in the wrapper to facilitate opening the package. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

181. Dried buttermilk improves palatability of reconstituted milk. G. H. Norman, 13th AF Central Exchange, APO 74, San Francisco, Calif. Milk Dealer, **44**, 4: 121. 1955.

Taste panel tests conducted on reconstituted milk plain, reconstituted milk with sweetened cream buttermilk, and fresh whole milk show that reconstituted milk plain was easily detected. Between the fresh whole milk and reconstituted milk with 15% sweet cream butter milk, the results were a 60-40 choice of fresh milk over reconstituted. When buttermilk powder used was lowered to 10%, the results were a 40-60 choice favoring the reconstituted over the fresh whole milk. The acceptance of this new reconstituted milk with spray-dried sweet cream buttermilk as part of the formula became evident when sales jumped from a normal 30,000 quarts per month to 168,000 quarts per month within a period of 3 months. Other tests showed that the use of buttermilk powder did not lessen the keeping quality of the reconstituted milk. C. J. Babcock

182. Casein-copolymeric styrene-maleic anhydride type compositions for and method of sizing textile warp yarns. E. H. Rossin (assignor to Monsanto Chemical Company). U. S. Patent 2,697,672. 15 claims. Dec. 21, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 3: 578. 1954.

A formula is given for a yarn sizing material consisting of a copolymer of styrene and maleic acid esters and casein in ratios ranging from 70:30 to 30:70 and used at temps. of 120 to 180° F. R. Whitaker

183. Process for preparing whey food. M. E. Hull (assignor to Armour and Company). U. S. Patent 2,699,995. 7 claims. Jan. 18, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 3: 421. 1955.

Partially delactosed whey liquor is standardized to about 30% total solids, adding from 5 to 40% by weight of an oleaginous material and sufficient sugar to prevent microorganismal spoilage and evaporating under vacuum to a total solids content of 70-80%. R. Whitaker

DAIRY BACTERIOLOGY

184. Some facultatively anaerobic gram-negative rods from the rumen of the calf and the sheep. E. S. M. Mackay and A. E. Oxford, Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol., **11**, 3: 472. 1954.

In the rumens of twelve calves from 10 to 105 days of age, the coliform population ranged from 10^5 to 10^6 per gram and consisted of mostly *Escherichia coli* and intermediate types rather than the saprophytic *Aerobacter* species. In contrast, capsulated strains of *Aerobacter aerogenes* could be isolated with relative ease from the rumen of a starch-fed sheep. A catalase negative non-coliform organism capable of hydrolyzing urea was isolated from the rumens of two older calves. J. J. Jezeski

185. The effect of ribonuclease on phage-host interaction. J. Kleczkowski and A. Kleczkowski, Rothamsted Expt. Sta., Harpenden, Hertfordshire. J. Gen. Microbiol., **11**, 3: 451. 1954.

Crystalline ribonuclease from pancreas was found to prevent permanent combination between a Rhizobium bacteriophage and host cells and thus inhibited the multiplication of the phage in liquid bacterial cultures. The addition of ribonuclease under conditions where the phage had already combined with the bacteria did decrease but did not completely prevent multiplication of the phage. However, the enzyme also interfered with multiplication of the bacteria. In experiments in which ribonuclease was used in connection with chymotrypsin it was suggested that phage and bacteria unite in the presence of ribonuclease but that the union was only transitory with the phage remaining active. In the presence of chymotrypsin the phage became inactive during this transitory union. J. J. Jezeski

186. Carbon dioxide utilization by rumen microorganisms. C. N. Huhtanen, F. J. Carleton and H. R. Roberts. J. Bacteriol., **68**: 749. 1954.

$Na_2C^{14}O_3$ was used as the source of CO_2. Incorporation of labelled CO_2 was followed by counts on selected fractions and by radioautograms. Of several bacteria tested, those producing lactic acid incorporated the most CO_2. Radioactivity was found in acetic, propionic, and $>C_4$ acids when RO-Cl was the test organism. Using RO-L5, radioactivity was found in aspartic acid, glutamic acid, threonine, phenylalanine, and arginine (absent in 12 other amino acids) and most of the remaining 35-40% of the activity was in the "nucleic acid" fraction. F. E. Nelson

187. The effect of methionine and methionine precursors on the growth of Streptococcus lactis. D. E. Kizer, M. L. Speck, and L. W. Aurand, N. C. State Coll., Raleigh. J. Bacteriol., **69**: 16. 1955.

Eight strains of *S. lactis* grew at a slightly reduced level when methionine was not added to the synthetic medium. Growth at a more reduced level occurred when methionine, serine, and glycine were deleted simultaneously. When serine was not deleted from the medium from which methionine and glycine had been omitted, homocysteine addition brought growth up nearly to the level characteristic of the complete medium. Addition of folic acid or vitamin B_{12} to the medium in which homocysteine had been substituted for methionine did not increase growth. The relationship of these results to those obtained on methionine synthesis in other organisms is discussed. F. E. Nelson

188. Diacetyl oxidation by Streptococcus faecalis, a lipoic acid dependent reaction. M. I. Dolin, Oak Ridge Natl. Lab., Oak Ridge, Tenn. J. Bacteriol., **69**: 51. 1955.

When ferricyanide is present as an electron acceptor, vacuum-dried cells of *S. faecalis* catalyze oxidation of one molecule of diacetyl to two molecules of acetate. When ferricyanide is not present, acetoin and diacetylmethylcarbinol accumulate as the reduced products. Cocarboxylase is essential for diacetyl oxidation by cell-free extracts. By the use of vacuum-dried cells deficient in lipoic acid, oxidation of diacetyl can be shown to be dependent upon lipoic acid. F. E. Nelson

189. A stereoscopic method for counting bacterial colonies (note). C. E. Clifton, Stanford Univ. School of Med., Stanford, Calif. J. Bacteriol., **69**: 107. 1955.

A stereoscopic microscope with 1x objective and 9x oculars was used. Light was predominantly transmitted, but some reflected light was used when the agar was opaque. The petri dish is placed on a Wolffhuegel plate, the two being moved simultaneously to permit colony enumer-

ation on defined areas. Under some circumstances counts may be made after incubating 5 or 6 hours. The characteristics of the colonies also may be noted. Bacteriophage plaque counts may be made if proper illumination is employed.
F. E. Nelson

190. Manometric estimation of rumen urease (note). C. N. HUHTANEN and L. S. GALL, Natl. Dairy Research Lab., Inc., Oakdale, L. I., New York. J. Bacteriol., **69**: 102. 1955.

The method is based upon the absorption of CO_2 from the gas phase by the NH_3 produced during hydrolysis of urea. The rumen fluid after gassing should be between pH 6.8 and 7.0. The reaction is carried out in usual Warburg apparatus. The urease activity of rumen fluid was more than equivalent to the urease activity of 2×10^9 cells/ml of *Proteus mirabilis*. This value is considerably greater than can be attributed to facultative bacteria in normal rumen fluid; probably the activity is associated with anaerobic microorganisms. F. E. Nelson

191. Influence of environmental factors on Streptococcus agalactiae isolated from active cases of bovine mastitis. J. H. GHOLSON and H. A. HERMAN, Mo. Agr. Exp. Sta., Columbia. Res. Bull. 534. 1953.

The optimum pH for growth of *S. agalactiae* in casein-acetate-lactose broth was 7.5. Cultivation at pH 6 or 8.3 resulted in the appearance of long chain forms. Long chain forms usually changed to short chains by continuous subculture of pH 7. Sodium acetate in a 15 mg/% concentration stimulated growth in broth, while 3% exhibited marked depressing effects. A combination of 15 mg/% of sodium acetate and 100 mg/% phosphates (equal quantities by wt. of mono- and di-potassium phosphate) provided optimum growth conditions. Sodium citrate alone or in the presence of sodium acetate exhibited a depressing effect on growth. A concentration of 10% lactose resulted in the formation of long chains, while 4% gave rise to short chain forms. Wide zone, beta-hemolytic colonies were typical of active cases of streptococcal mastitis in the station dairy herd. Continuous subculture of *S. agalactiae* in casein-acetate-lactose broth gave rise to a variant similar to that resulting from mouse and rabbit passage. Streptococci possessing enhanced mouse virulence do not necessarily have an increased toxicity for bovine udders. Not all short chain forms of beta-hemolytic Group B streptococci show toxic components but all those which did exhibit toxic components were short chain forms. R. W. Hunt

192. Bacteriology of milk and milk products. Nature, **174**, 4442: 1135. 1954.

Five papers given at the Oct. 26, 1954 meeting of the Society for Applied Bacteriology in London are reviewed. Subjects discussed include coliform organisms in dairy water supplies and in milk, the laboratory and field evaluation of chemical sterilizers for dairy farms, the bacteriology of sterilized milk, and the development and uses of nisin.
R. Whitaker

DAIRY CHEMISTRY

193. Goitre and the iodine content of cows' milk. W. T. BINNERTS. Nature, **174**, 4438: 973. 1954.

The iodine content of milk in four sections of the Netherlands varied considerably. Milk from the goitrous section was low in I, whereas the I content of milk from non-goitrous and the extreme coastal areas was approximately three times greater. Summer milk was about twice as rich in I as winter milk. R. Whitaker

194. Present status of the foreign fat detection problem. M. KEENEY, Univ. of Md., College Park. Ice Cream Rev., **38**, 6: 85. 1955.

Advantages and limitations of several proposed tests for the detection of foreign fats are discussed. These include the sterol acetate, tocopherol, butyric acid, critical temperature of dissolution tests, the Reichert-Meissl, Polenske numbers and refractive index.

The detection of 5 to 10% adulteration with vegetable fat is legally possible through the use of a combination of tocopherol analysis, the Polenske number and sterol acetate tests. In the case of adulteration with animal fats, an adulteration of 20% is the minimum which could be successfully prosecuted in courts. The hope is expressed that upon completion of a study now in progress on a nationwide basis a 10% adulteration with animal fats may be detected with legal proof. W. J. Caulfield

DAIRY ENGINEERING

195. Can washing machine. N. J. PETERS (assignor to Damrow Brothers Company). U. S. Patent 2,698,624. 7 claims. Jan. 4, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 1: 54. 1955.

Construction details are given for a milk can washer of the straight line type. Two rows of cans may be washed in the same machine, a switching platform directing the cans alternately from one conveyor line to the two rows.
R. Whitaker

196. Centrifugal separator. J. E. COOK (assignor to The De Laval Separator Company). U. S. Patent 2,698,131. 5 claims. Dec. 28, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 4: 759. 1954.

A conventional-type milk separator is described which has the motor totally enclosed within the frame and located to the side of the bowl spindle, rather than directly below the bowl. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

197. Dairy marketing in the Northern Great Plains—its patterns and prospects. E. FEDER and S. W. WILLIAMS, S. D. Agr. Expt. Sta., Brookings. Bull. 438. 1954.

A study was made in respect to the future of dairying in the 4 Plains states (N. D., S. D., Neb., and Kan.). Major conclusions are: (1) Unless there are major changes in technology, dairying is likely to remain a sideline enterprise on a high proportion of farms in the area. Physical and economic conditions favorable to grain and beef are likely to result in further declines in milk production. (2) Periods of low farm income may result in increased sales of milk and particularly of cream. (3) These four states will continue to produce an appreciable proportion of creamery butter of the nation. (4) Further shifts from farm-separated cream sales to whole milk will be limited. (5) Government farm programs are not likely to alter the dairy industry of the region. R. W. Hunt

198. Input-output relationships in specialized butter-powder and cheese plants. G. T. NELSON, Ore. Expt. Sta., Corvallis. Tech. Bull. 32. 1954.

This report was written to show some of the fundamental economic relationships of plant operations. The information is considered for use by management as a guide in considering the consolidation of small plants, planning a new plant, or measuring the efficiency of an existing plant. Supply cost is the most important cost item of both roller and spray plants; 45% of the manufacturing cost of the roller plants and nearly 50% for the spray plants. Labor is higher than capital costs for roller plants but lower for spray plants because of the high capital cost of spray drying equipment. Total manufacturing costs per 1,000 pounds of milk for 1952 range from $6.42 to $7.77 for butter-roller powder, $6.23 to $9.51 for butter-spray powder, and $4.68 to $8.51 for cheese plants. Lower supply and capital costs account for lower unit costs of manufacturing milk into cheese than when milk is made into butter, and either roller or spray process powder, in plants of comparable size.
R. W. Hunt

199. Insurance for the ice cream plant. M. K. DOEHLER, JR., Bruce Dodson and Co., Kansas City, Mo. Ice Cream Rev., **38**, 6: 46. 1955.

This article discusses in detail the types of insurance needed by the average dairy company, and includes suggestions for reducing fire insurance rates and for minimizing insurance costs. W. J. Caulfield

200. The high cost of delivery. T. D. LEATHERS, Milk Plant Monthly, **44**, 1: 13. 1955.

Delivery of a quart of milk to the greater Cleveland consumer costs about 1/3 of the current retail price of 21¢. Wages for the retail driver and the upkeep on and the replacement of his truck constitute the greatest part taken out of the consumer's dollar. The retail delivery man draws wages and commissions averaging more than $500 a month or about $2.40 an hour. Surveys that have attempted to analyze farm costs of milk allow the dairy farmer about $.90 an hour for his wages. The driver's share of the price of quart of milk is 3½¢. This cost is increased to 3.8¢ by replacement driver wages, and fringe benefits under the union contract hike the total labor delivery cost to 4.2¢. Wholesale delivery costs are about 2¢ a quart. C. J. Babcock

201. Meeting milk's competition in school cafeterias. W. M. ALLRED, Utah State Coll., Logan. Milk Dealer, **44**, 4: 50. 1955.

Milk can compete successfully with soda pop in schools where students have free choice of both at the school lunch counter if milk is properly priced and merchandised. If it is not, milk runs a poor second. For example, in school year 1948-1949 when milk was sold in glass and priced at 7¢ per half pint, 5.7 bottles of soda pop were sold for each bottle of milk. However, in 1950-1951 when milk was priced at 5¢ and no-deposit paper cartons were introduced, an equal number of containers of milk and chocolate milk and soda pop was sold. Other data in tabular form are presented.
C. J. Babcock

202. Carrier for dairy products. A. D. MALLIARIS. U. S. Patent 2,697,540. 2 claims. Dec. 21, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 3: 545. 1954.

A hand carrier made of wire for 8 bottles of milk. R. Whitaker

203. Bottle carrier. P. J. BURKE and C. LIETZAU. U. S. Patent 2,697,628. 4 claims. Dec. 21, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 3: 569. 1954.

A wire grip and handle for carrying two milk bottles. R. Whitaker

FEEDS AND FEEDING

204. Effects of carbohydrate feeding levels on roughage digestion in dairy cattle. C. F. FOREMAN and H. A. HERMAN, Mo. Agr. Expt. Sta., Columbia. Research Bull. 535. 1953.

Digestion trials were run with dairy cattle to investigate effects of carbohydrate level (molasses) on digestibility of constituents of roughages. A study also was made of types and numbers of micro-organisms normally found in the bovine rumen. Counting of protozoa was restricted to those resembling ciliates.

No relationship could be observed between make-up of ration and number of protozoa counted. Bacterial numbers in the rumen in-

creased as the amount of molasses fed daily was increased. Increases in the apparent digestibility of crude protein, cellulose, and crude fiber were obtained when daily feeding levels of up to 2 pounds of molasses were fed. When the roughage was mixed timothy-lespedeza hay, 4 pounds of molasses fed daily decreased digestibility of crude protein, cellulose, and crude fiber. Nitrogen-free extract digestion was increased with the increase in daily molasses feeding levels. R. W. Hunt

205. Feed digestibility. Chemical method for measuring relative digestibility of animal protein feedstuffs. A. J. GEHRT, M. J. CALDWELL, and W. P. ELMSLIE, Moorman Manufacturing Co., Quincy, Ill. J. Agr. Food Chem., **3**: 159. 1955.

A simple enzymatic method is useful in determining the relative digestibility of meat and fish by-products used as feedstuffs. The indigestible residues may be studied microscopically for identification of most of their constituents which consist predominantly of vegetable fiber, hoof, colloidal organic matter, hair, fuzz, and charred meat in various proportions. Indigestible residues in meat scrap range from 2 to 25%, seldom exceeding 10% in samples of good quality. Limited studies on fish meal show a high degree of digestibility unless the product has been overheated. Blood meal is highly digestible when properly processed, but may be much less digestible if overheated in the drying process. Reproducibility of the method is good, with less than 1% difference between duplicate samples. S. Patton

GENETICS AND BREEDING

206. Observations on the ovaries of infertile and reportedly infertile dairy catle with reference to the pathologic aspects. J. F. BONE, Ore. Expt. Sta., Corvallis. Tech. Bull. 30. 1954.

Genital tracts of 55 dairy cows slaughtered for infertility reasons are described. Inadequate professional services resulted in the needless sacrifice of 31% of the slaughtered animals while the cysts associated with corpora lutea were present in 14% of the nonpregnant cattle. Several other findings were made.

R. W. Hunt

207. The recovery of Trichomonas fetus after freezing to −79° C. in prepared milk semen extender. D. H. MCWADE and J. A. WILLIAMS, Mich. State Coll., East Lansing. Quart. Bull. 37, 248. 1954.

Because of increasing use of frozen semen in artificial insemination during this past year and the possibility of the spread of trichomoniasis from infected semen, it was felt advisable to investigate the effect of deep freezing as a possible method of destroying *T. fetus*. Recovery of *T. fetus* was made from both plain milk and glycerinated milk which has been frozen to −79° C. and held at this temperature up to 125 days. R. W. Hunt

HERD MANAGEMENT

208. Profitable milk production from grazing crops, Piedmont area, South Carolina. C. P. BUTLER, S. C. Agr. Expt. Sta., Clemson. Bull. 411. 1953.

Farms containing 30 to 99 acres of cropland occupy a major portion of the tillable land in the Piedmont area of S. C. A medium size farm consisting of 74 acres of cropland and 14 acres of open pasture was selected for this analysis. This farm had a net income of less than $1000 in 1951. Three alternate systems of farming were analyzed for this representative farm: (1) an improved cash crop system, (2) a cash crop system combined with a 10-cow dairy, and (3) a 30-cow grade "A" dairy. The result of the analysis showed that returns to labor and management on a representative medium-sized farm could be $6500-$7000 for the Grade "A" 30-cow farm, the cash crop systems would produce returns of about $1700, and the 10-cow dairy enterprise would return a little over $1900. Many farms are now using the adjustment practices recommended as a result of this study. R. W. Hunt

209. Use of the lounge area by dairy cows in loose-housing when self-fed roughage in an outside lot. R. C. LEWIS and J. D. JOHNSON, Mich. State Coll., East Lansing. Quart. Bull. 37, 218. 1954.

A Brown Swiss herd in loose-housing was observed, to study their usage of the lounge area when hay and silage or hay alone was self-fed in the paved lot. Traffic in and out of the lounge area was about evenly divided between day and night. Although the cows spent more total time in the lounge during hours of darkness, the time spent in the lounge during the daytime was about evenly divided between loitering and resting, while at night ¾ of the time was spent in resting. Cows spent more total time in the lounge when hay was fed. Hay alone appeared to be slightly more effective than hay and silage in inducing the animals to leave the lounge area immediately after milking, although a large percentage of the cows returned directly to the lounge especially after the morning milking. R. W. Hunt

210. Automatic animal duster and back scratcher. C. N. PETERSON. U. S. Patent 2,697,415. 2 claims. Dec. 21, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 3: 513. 1954.

To a frame mounted on posts and supporting a trough of dusting powder, are attached two arms. A number of chains, stretching between the arms and the supporting posts, provide a structure against which cattle rub their sides

and back. The movement of the animal pulls the chains, which in turn move the arms and release the dusting powder. R. Whitaker

211. Milking machine pail lid. L. F. BENDER (assignor to National Cooperatives, Inc.). U. S. Patent 2,697,412. 8 claims. Dec. 21, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 3: 512. 1954.

The pulsator for a vacuum milker is mounted on the top of the lid of a milk pail.
R. Whitaker

212. Portable barn gutter cleaner. R. G. FERRIS and R. L. YUENGER (assignors to Starline, Inc.). U. S. Patent 2,698,105. 21 claims. Dec. 28, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 4: 752. 1955.

A device for removing refuse from barn gutters to a designated area outside the barn.
R. Whitaker

213. Cattle working gate. L. A. PFULLMAN. U. S. Patent 2,698,601. 4 claims. Jan. 4, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 1: 48. 1955.

A gate is described with an attachment for restraining the head of cattle. R. Whitaker

214. Standpipe type milk releaser. H. A. HECKENDORF (assignor to International Harvester Company). U. S. Patent 2,699,145. 9 claims. Jan. 11, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 2: 197. 1955.

An arrangement of valves and standpipes attached to a milk reservoir on a vacuum-type milking machine, which permits the discharge of the milk to atmospheric pressure from the vacuum system. R. Whitaker

215. Dehorner. B. A. SEARS. U. S. Patent 2,699,783. 7 claims. Jan. 18, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 3: 367. 1955.

A device for clipping the horns of cattle by means of hydraulically-operated knives when attached to the head of the animal.
R. Whitaker

216. Calf's nipple. A. T. EZELL (assignor to Ezell Sales, Inc.). U. S. Patent 2,699,778. 1 claim. Jan. 18, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 3: 366. 1955.

A nipple for feeding young calves so designed that it fits over the lip of a container, such as a milk bottle. R. Whitaker

ICE CREAM

217. Will she buy your brand? F. A. BABIONE, Penn. State Univ., State College, Pa. Ice Cream Field, **63**, 4: 56. 1954.

A marketing survey made by Penn. State Univ. revealed that 82% of the women plan to buy ice cream before entering the store. Sixty-four % of the women serve ice cream at least once each week, and 63% would not go out of their way to buy a preferred brand. Convenient location clearly overshadows brand as a basis of purchase. Only 10% of the women bought ice cream on a price basis.

Only one-third of the women considered insulated bags of importance. The distance from the store is the chief consideration in wanting them. Hand-dipped ice cream was preferred by 45% of the women; factory-packed by 35% and 20% had no strong preference.

Television ads were remembered more than all other types of advertising combined—five times as much as radio and more than 10 times as much as newspaper ads. W. C. Cole

218. Research aids quality and sales in Georgia. H. B. HENDERSON, Univ. of Ga., Athens. Ice Cream Field, **64**, 6: 80. 1954.

Clinics have substantially improved ice cream quality in Georgia. Research has aided in reducing shrinkage, in establishing the most effective stabilizers and emulsifiers, and in determining the extent to which milk fat may be adulterated with foreign fats. Plastic ice cream cartons, treated cartons, and untreated cartons are listed in the order of preference for storing ice cream. W. C. Cole

219. Low carbohydrate ice cream for diabetics. C. G. ROBERTS and C. D. DAHLE, Penn. State Univ., State College. Ice Cream Field, **63**, 3: 52. 1954.

Two basic mix formulas are given for commercial preparation of low carbohydrate ice cream. Flavors other than vanilla can be used provided they are sugar free or have been considered in estimating the insulin demand of the ice cream in which they appear.

A sorbitol solution is used in these formulas to replace sucrose and partially sweeten the ice cream. Sucaryl and saccharin are used as supplemental sweetening agents. W. C. Cole

220. Fat-smoothness research. W. S. ARBUCKLE and L. F. CREMERS, Univ. of Md., College Park. Ice Cream Field, **64**, 4: 98. 1954.

Results of a microscopic study of the distribution of fat globules in ice cream are presented. Thin sections of ice cream were immersed in various liquids selected on the basis of their refractive indexes. It was observed that fat globules tended to accumulate in chain-like arrangements around the air cells and throughout the unfrozen material. The higher the overrun in the ice cream, the more distinct was the chain-like arrangement of the fat globules. W. C. Cole

221. Direct-draw milk shakes study. J. J. SHEURING, Univ. of Ga., Athens. Ice Cream Field, **64**, 4: 106. 1954.

Experiments designed to evaluate various types of mixes for direct-draw milk shakes are reported. Increasing the solids in the mix and lowering the drawing temperature of the shake

contribute towards a thick cold shake preferred by consumers.

Compositions and criticisms are tabulated for 10 typical mixes. Instead of making special mixes for direct-draw shakes, satisfactory results were obtained by diluting standard ice cream and ice milk mixes in the proportion of 1 part mix to 1 part homogenized milk.

W. C. Cole

222. Your "other" products—A study of state laws regulating their sales. ANON. Ice Cream Field, **63**, 5: 28. 1954.

Special consideration is given to ice milk, mellorine, and diabetic ice cream. A brief statement is made for each state and a tabulation included which gives the legality of manufacturing each of these products in all of the states.

W. C. Cole

223. What happens to ice cream in self-service cabinets. W. B. COMBS and E. L. THOMAS, Univ. of Minn., St. Paul. Ice Cream Field, **64**, 6: 34. 1954.

This progress report deals with factors affecting the keeping quality of ice cream stored in self-service cabinets and home freezers.

Soft ice cream, especially in the top layers of packages in self-service cabinets, and subjected to temperature fluctuations in cabinets, markedly increased in coarse texture when stored for periods of 1 week to 1 month. Accumulation of ice on exterior of packages also resulted from long storage.

Coding and proper rotation of packages may aid in preventing quality deterioration. Adequate temperature control can be achieved thru improvement in certain mechanical features of cabinet design. W. C. Cole

224. Sanitary cover for stirrers of milk shaking machines. B. J. ROSENTHAL. U. S. Patent 2,698,165. 5 claims. Dec. 28, 1954. Offic. Gaz. U. S. Pat. Office, **689**, 4: 769. 1954.

A flexible cup-shaped cover for providing sanitary protection for the stirrer of a milk-shake machine. The cup is mounted on a flexible shaft, the other end of which is attached to the base of the machine by means of a spring. The cover is placed on and off the mixer by bending the shaft and spring. When the mixer is in use, the shaft holds the cover in a vertical position to the side of the mixer.

R. Whitaker

225. Machine for filling and capping ice cream cups. J. A. DUKE. U. S. Patent 2,699,281. 6 claims. Jan. 11, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 2: 232. 1955.

An automatic volumetric filler.

R. Whitaker

226. Coliforms in fruit ice cream. F. W. BARBER and H. FRAM, Natl. Dairy Research Labs., Inc., Oakdale, Long Island, N. Y. Ice Cream Field, **65**, 1: 78.

During the past year the authors have shown that there are present in strawberry, peach, and banana purees bacteria which give a false positive coliform test on ice cream made with these purees. For the most part, the bacteria involved are Gram-negative short rods, but some Gram-positive cocci and a few yeasts have been encountered. All of the organisms studied were indistinguishable from true coliform bacteria in solid media tests and sometimes in liquid media tests in the presence of ice cream solids. Sucrose is likely responsible for the development of large red colonies on the solid test media and the production of gas in the liquid test media. A procedure for differentiating them from true coliform bacteria is outlined.

With one exception, the false positive coliform reaction has been found only in fruit ice cream. W. C. Cole

227. How important are coliform tests. F. W. BARBER, Natl. Dairy Research Lab., Inc., Oakdale, Long Island, N. Y. Milk Plant Monthly, **44**, 1: 18. 1955.

The coliform test is of value as a quality control measure and as a technique for the evaluation of sanitation and sterilization procedures. The results must be correctly interpreted or the tests will mean little. Standards, methods of conducting the tests, and the importance of false positive coliform results, especially in fruit ice cream, are discussed.

C. J. Babcock

MILK AND CREAM

228. Factors relating to the formation of a fat-ring on homogenized milk. B. J. DEMOTT, G. M. TROUT, and J. R. BRUNNER, Mich. Agr. Expt. Sta., East Lansing. Food Research, **19**, 6: 603. 1954.

The "cream ring" sometimes forming at the tops of bottles of homogenized milk consists of separated fat in a concentrated condition. The defect was related to low temperatures of homogenization and high fat levels in milk. The addition of extra solids in the form of dry skimmilk was not found to be a deterrent but higher than normal temperatures of homogenization were. F. J. Doan

229. Container and dispensing means for a pressurized food product. F. H. APPEL, A. E. ACKER, and D. F. WELCH (assignors to Pressure Containers, Inc.). U. S. Patent 2,699,890. 14 claims. Jan. 18, 1955. Offic. Gaz. U. S. Pat. Office, **690**, 3: 393. 1955.

A container and release mechanism is described for liquid foods, such as cream, packed under pressure. R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

230. Cinefluorographic studies of the ruminant stomach and of eructation. R. W. Dougherty and C. D. Meredith, Cornell Univ., Ithaca, N. Y. Am. J. Vet. Research, **16**, 58: 96. 1955.

A fluoroscopic apparatus adapted for taking motion pictures was used to study the belching activity of sheep. Rumen fistulated sheep were used to observe the differences in belching in partially filled and overfilled stomachs. Even with serious overfilling, contractions of the rumen and reticulum were able to clear ingesta from the cardia. Relaxation of the esophagus at the same time allowed gas from the rumen to enter easily and to be expelled quickly. It was difficult to cover the cardia with fluid. These studies suggest that bloating may not be produced easily by simple mechanical overfilling of the rumen, because that cannot prevent eructation. E. W. Swanson

231. Changes in temperature of reticuloruminal content following the drinking of water. R. D. Dillon and R. E. Nichols, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **16**, 58: 69. 1955.

The temperature changes of the cardia, reticulum, and ventral rumen contents of a cow following the consumption of water were recorded by means of rods fitted with thermocouples. Water varying from 10 to 54 lb. and from 36 to 60° F. was consumed by the cow in different trials. The cardia, reticulum, and rumen dropped in temperature in that order and recovered to normal in the same order. The rumen temperature remained below 90° F. for 45 min. after the consumption of 54 lb. of water, and 44 lb. of 36° F. water kept it below 90° F. for 43 min. The rumen was still below 95° F. 110 min. after consuming the 50° F. water. Only slight changes were produced by 10 or 20 lb. of 60° F. water.

E. W. Swanson

232. Plasma and milk tocopherol levels of cows compared with the plasma tocopherol levels of their foster calves. J. W. Safford and K. F. Swingle, Mont. Agr. Expt. Sta., Bozeman. Am. J. Vet. Research, **16**, 58: 64. 1955.

Three cows whose calves had developed muscular dystrophy were given to nurse healthy male Jersey calves from a herd not affected with muscular dystrophy. Tocopherol levels in blood of cows and calves and in milk were determined over a 100-day period. None of the Jersey calves developed muscular dystrophy. Milk tocopherol averaged 41.7, 26.8, and 42.5 μg. per 100 ml. for the three cows, and did not rise when the cows were turned into pasture even though their blood tocopherols rose markedly. During the hay-feeding period the cows' blood tocopherols averaged 252, 244, and 231 μg. per 100 ml., and the calves averaged 78.6, 37.4, and 21.4 μg. per 100 ml. There seemed to be no correlation with blood and milk tocopherol levels of the dams and the blood levels of the calves or the occurrence of muscular dystrophy in the calves. E. W. Swanson

233. New studies of dairy cow production. J. C. Shaw, Univ. of Md. Milk Plant Monthly, **44**, 1: 26. 1955.

The value of using radioisotopes in studies with animals is discussed. C. J. Babcock

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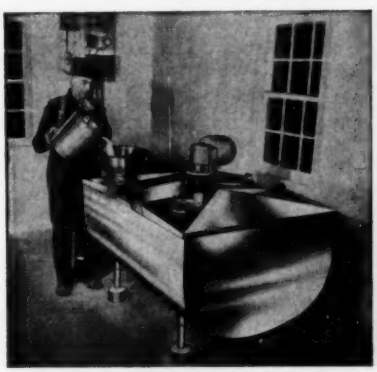

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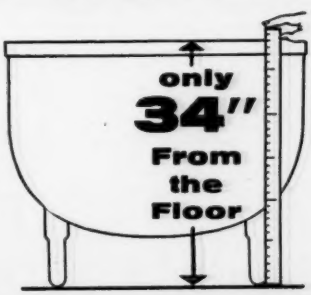

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